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EDITED BY

JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

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THE
BOTANICAL GAZETTE

April 1922

DEVELOPMENTAL SELECTION IN VASCULAR PLANTS[1]

JOHN T. BUCHHOLZ

(WITH TWENTY-EIGHT FIGURES)

In the numerous explanations and discussions of natural selection in the *Origin of species* and since the time of DARWIN, the process of competition has usually been regarded as taking place in the external environment. In striking contrast with this, developmental selection is characterized by the fact that it occurs between very minute or embryonic individuals whose struggle is limited to what might be termed an internal environment. It is well illustrated by the selection resulting from the polyembryony within the developing seeds of conifers and cycads, the embryonic selection in this case being a special form of developmental selection. The embryos of the latter are wholly surrounded by organic tissue; they are entirely inclosed within the ovule of the parent plant. Equivalent forms of developmental selection are found in ferns as well as in angiosperms, and it is intended to discuss briefly these internal selective processes in their relation to organic evolution.

Developmental selection is not to be confused with any of the older well known theories involving internal forms of selection. WEISMANN'S germinal selection is described as an internal process, but this is a supposed struggle between biophores within the germ cells; it is not even a competition between individual cells, and can be imagined only. It is based on a speculative hypothesis

[1] Presented before the Botanical Society of America, Chicago, December 29, 1920.

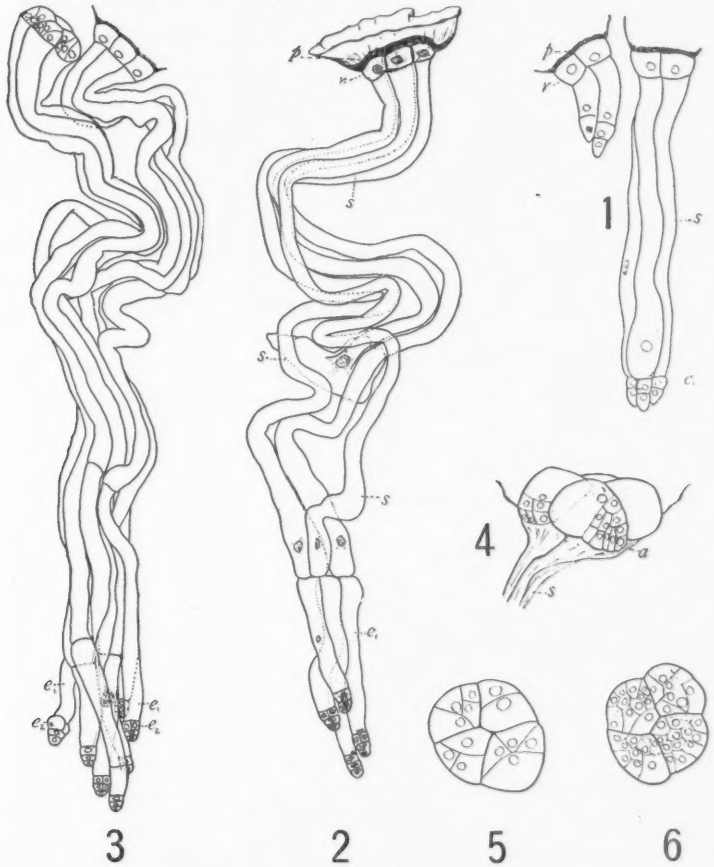
entirely incapable of experimental proof. Likewise ROUX's theory of intra-selection or the battle of the parts, a supposed struggle between the various organs of the body of a developing individual, is not a struggle between different individuals, but only between differentiating tissues and organs. Developmental selection, on the other hand, occurs between definite individuals which may be unicellular or multicellular, but the process is not intracellular. This can easily be demonstrated and is capable of being subjected to observation and experimental study.

The several isolation theories, as well as the other theories of species forming auxiliary to natural selection, have neglected any significant allusion to the type of illustrative material discussed in this paper. Developmental selection is different also from the theories of sexual isolation, physiological selection, or mechanical selection.

A definite rôle is not to be denied for natural selection, but it is not the only selective process. In developmental selection, we have a supplementary form of selection which occurs at other times during the life cycle, one which meets some of the most serious objections which have been raised against natural selection. It is capable of playing on mutations as well as other forms of variation, bringing about definite results in evolution. In fact, a real species forming rôle may be claimed for developmental selection, if we grant that such a rôle is to be found in any selective process. During the ontogeny of higher plants, therefore, there are a number of stages in the life cycle when a definite competitive selection between individuals occurs. In addition to natural selection, there is also this definite struggle between supernumerary gametophytes, when these are dependent on sporophytes, between supernumerary embryos as found in the polyembryony of gymnosperms and most ferns, or between excessive numbers of gametes.

In plants, natural selection, as it is ordinarily understood, occurs in the environment, when seeds or spores germinate in or on the soil, or when vegetative organs, such as roots, rhizomes, stolons, etc., from several neighboring plants of similar or different species give rise to new individuals in a crowded stand and in close competition. For animals, natural selection is usually understood to begin at birth, or when the young first come into contact with

the external environment. That a selection may occur during the earliest developmental stages of an individual, for instance, during



FIGS. 1–6.—Embryos of *Pinus Banksiana* showing embryonic selection: fig. 1, two neighboring archegonia giving rise to embryos; fig. 2, system of eight embryos and their suspensors (*s*, *e*) derived from single zygote by cleavage (rosette embryos [*r*] still unicellular, but shown in later stages in figs. 4–6); fig. 3, two embryo systems produced by fertilization of two neighboring archegonia, with sixteen embryos, eight primary embryos (below), and eight rosette embryos (above), participating in the competition.

the embryology or during the processes of seed development, is an idea that apparently has not been considered seriously by

students of biology, judging from the general neglect of the subject of polyembryony in the literature of organic evolution. Many of the important facts concerned in the process of embryonic selection in gymnosperms, as the polyembryony of conifers and cycads, which have not been linked up with evolutionary doctrine, have been known to science for nearly a century.

Besides being entirely inclosed within the tissues of the parent plant, developmental selection is characterized by being intraspecific or reflexive. It is always a definite competition between similar individuals; usually it is between those of a single fraternity.

NATURAL SELECTION

Environmental process occurring in external physical and biological environment of organism, where conditions of struggle for existence are very complex

- Struggle against unfavorable environment of physical surroundings.
- Struggle against other species; extraspecific competition.
- Struggle against fellows; intraspecific competition.

DEVELOPMENTAL SELECTION

Occurring during early embryonic or gametophytic stages within tissues of parent plant, under conditions uniform for competing individuals

- Selection between vegetatively branching parts of either the gametophyte or sporophyte; buds and branches of trees, which later give rise to reproductive parts.
- Interovular selection, between ovules within same ovary: (1) after fertilization, largely due to activities of contained embryos; (2) before fertilization, due in part to activities of contained female gametophytes, megaspores, or archesporial cells.
- Embryonic selection, between embryos within the same ovule, or within tissues of parent gametophyte.
- Gametophytic selection: (1) between male gametophytes, such as pollen tubes within carpellary and nucellar tissue; (2) between female gametophytes within the same ovule.
- Gametic selection: (1) between male gametes or sperms; (2) between female gametes or eggs.

Although it is realized that in many instances this developmental process is influenced by external conditions, it is clear at least that the influence of these environmental conditions is very indirect, and that the highly complex external environment does not exert

a differential effect on the competing individuals. The foregoing outline is suggestive of the general relation of the several forms of developmental selection to the general process of natural selection.

Developmental selection expresses itself in some form or other in the sexual reproductive cycle of practically all vascular plants. It is probably also involved in the life cycle of most of the cryptogamic forms, and is a factor to be reckoned with among animals as well. The main purpose in this discussion is to describe in a general way the various expressions of the principle of developmental selection as it applies to vascular plants.

The ordinary details of conifer embryogeny have been described (11) and may be assumed to be fairly well understood by botanists. It is generally known that in cycads or in such conifers as the spruce, for example, there are several embryos that engage in an intense life and death competition during their development. Only one embryo reaches its full term of growth to become the seed embryo, while the weaker individuals are aborted in the earlier stages. Only in extremely rare cases may two embryos be matured together in the conifer seed. In *Ginkgo* this happens rather more frequently, about 2 per cent of the seeds having been found with equally developed "twin" embryos (13). Even if several embryos should occur within the same testa, as in citrus seeds and several other angiosperms, these must necessarily come up so close together that a close competition between them will be inevitable after the seeds germinate. This competition which occurs after seeds germinate in the soil is environmental, however, and belongs to the categories of natural selection, where it remains as an intense intraspecific form of natural selection.

When pollination is successful enough to provide a plurality of male gametophytes, making polyembryony possible, practically all gymnosperms possess the feature of embryonic selection. Here the female gametophyte tissue is well formed before the embryos begin to develop, is somewhat firm and resistant, and it is only by a vigorous growth and rapid suspensor elongation, together with an abundant secretion of digestive ferments, that the successful embryo matures at the expense of its fellows and brings about their destruction. In angiosperms the endosperm within which the

embryo develops is much later in its origin, and is usually very soft and gelatinous in these early stages. This and the reduced suspensor of angiosperms may largely account for the fact that angiosperm polyembryony does not usually result in the definite selection of a single embryo before the seed is shed.

BROWN (1), who discovered polyembryony, pointed out that plurality of archegonia makes possible the fact of polyembryony among both cycads and conifers. It has been found also (**5, 35**) that in some conifers the zygote may undergo cleavage, resulting in several young embryos which compete with each other. Thus the fertilization of only one egg in *Pinus*, for example, results in the formation of eight embryos by cleavage (cleavage polyembryony), only one of which survives and completes its term of development (figs. 1–6). In other conifers, as in the spruce, the egg gives rise to only one embryo, but in any event the plurality of eggs makes possible simple polyembryony, in which a selection of embryos occurs.

A scheme of phylogeny, based in part on the character of polyembryony, whether simple or by cleavage, has been outlined in previous papers (**5, 6**). All the facts at hand seem to indicate that practically all conifers which do not possess cleavage polyembryony show structural evidence of having passed through this condition in their phylogeny. This indicates that either cleavage polyembryony originated among ferns, or it originated during the transition to the seed habit. All evidence is in favor of the latter alternative, and a definite hypothesis to account for the origin of cleavage polyembryony will be outlined in a later paper. In general, cleavage polyembryony is well developed among the more primitive conifers, and was eliminated sooner or later in all but one or two phyletic lines. Whether cycads passed through a similar stage of cleavage polyembryony is very uncertain. Nothing in the embryogeny of cycads thus far described appears to suggest this, but their simple polyembryony is doubtless of the same fern origin as that of conifers, that is, plurality of archegonia in the ferns from which cycads were derived.

Embryonic selection, either through cleavage polyembryony, plurality of archegonia, or a combination of both, is practically

universal among gymnosperms, and its character or type is of considerable importance in a study of phylogeny. For the origin of simple polyembryony, we must turn to a study of pteridophytes, as it is very evident that the plurality of archegonia in gymnosperm gametophytes was derived from a similar plurality among their pteridophyte ancestors.

Embryonic selection among pteridophytes

Many living pteridophytes have simple polyembryony, that is, a plurality of young sporophytes growing on a single gametophyte. Whenever the number of these sporophytes greatly exceeds the ability of the gametophyte to nourish all of them through their period of embryonic development, so that some of them are starved out in their early stages, a selection must occur among them. If this selection takes place in the earliest stages, before these young sporophytes are exposed to the external environment and become independent, we have embryonic selection as truly as that found in gymnosperms. When the several embryos in this competition are the result of a simultaneous fertilization, this embryonic selection has all of the advantages, as a measure of merit, that may be found in the gymnosperm polyembryony, and will be certain to result in the survival of the embryos that are strongest and most vigorous as determined by their actual performance. The facts that are definitely known concerning polyembryony and embryonic selection in various groups of pteridophytes may be considered as follows.

LYCOPODIALES.—The occurrence of several embryos per gametophyte in *Lycopodium* was definitely reported and shown by BRUCHMANN (3). Figs. 7–9 definitely show this plurality of embryos. These embryos do not all mature, but some of them remain in an arrested but viable condition for a considerable period, and are able to resume their growth if the larger sporophytes are injured by drought or otherwise. The smaller embryos probably fall into two groups, those which owe their origin to a fertilization simultaneous with that forming the successful embryo, and those which originate by a subsequent fertilization. It is very evident that the conditions for fertilization, even in these subterranean gameto-

phytes, are not continuous, but only occasional in occurrence. There is nothing that would hinder the fertilization of several or all of the archegonia which open on any occasion when fertilization takes place. A competition for food, together with a difference in the growth vigor of the embryos, probably determines which of several zygotes shall become the successful sporophyte. Some of the unsuccessful embryos doubtless abort and collapse in very

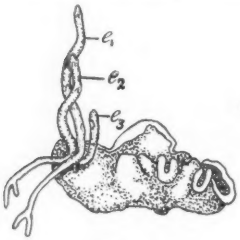


FIG. 7

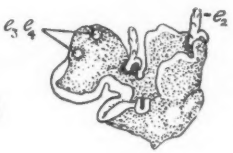


FIG. 8

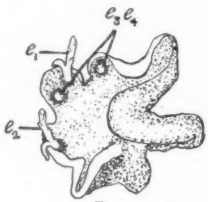


FIG. 9

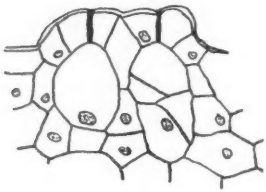


FIG. 10

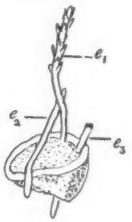


FIG. 11

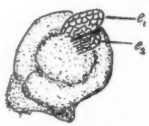


FIG. 12

FIGS. 7–12.—Fig. 7, gametophyte giving rise to several young sporophytes (e_1,e_2,e_3) of *Lycopodium annotinum*,×3; figs. 8, 9, gametophytes of *Lycopodium clavatum*, each with four embryos (e_1, e_2, e_3, e_4); after BRUCHMANN (**3**); fig. 10, section of gametophyte of *Tmesipteris* showing two neighboring embryos,×150; fig. 11, gametophyte of *Lycopodium volubile* bearing three sporophytes,×5; fig. 12, same with two very young sporophytes emerging,×8; figs. 10 and 11 after HOLLOWAY (**23, 24**); fig. 12 after CHAMBERLAIN (**10**).

early stages, soon becoming unrecognizable; while according to BRUCHMANN'S accounts, confirmed by subsequent observers, many of them remain in an arrested but viable condition for some time. These may doubtless be added to by subsequent fertilization of still other archegonia. It appears that among some *Lycopodium* species with large gametophytes several full fledged sporophytes may be produced.

CHAMBERLAIN'S (10) description of several New Zealand *Lycopodium* gametophytes includes two instances among his figures showing a plurality of young sporophytes. His figure of *L. laterale* shows one sporophyte with protocorm and two protophylls, while a second embryo of much smaller size has just broken through the gametophytic tissue. *L. volubile* (fig. 12) is also shown with two young sporophytes, one nearly twice the size of the other.

HOLLOWAY (23, 24) has made an extensive study of New Zealand *Lycopodium* and *Tmesipteris* gametophytes, and has described a number of them. *Tmesipteris* has polyembryony, it is figured twice with two very young embryos, one of which is reproduced in fig. 10, and *Lycopodium* species are frequently shown with several sporophytes per gametophyte (fig. 11). From HOLLOWAY'S letter, received in reply to an inquiry as to the occurrence of polyembryony among the New Zealand species of *Lycopodium* and *Tmesipteris*, the following paragraphs summing up these facts are taken:

> I have examined a large number of prothalli of *Tmesipteris* (most of them externally only), and have observed that not a few (I cannot say how many) bore two and even three well grown young plants on the same prothallus. These plantlets were developing healthily, and presumably would all continue to do so as the prothallus decayed away. Probably, of course, the time would then come when they would begin to crowd each other out. . . . . In the prothalli which I sectioned, I found two instances in which two archegonia side by side had been fertilized and were continuing their development. On one of these prothalli there was also the remains of the foot of an older plantlet. No other embryos were to be seen on these prothalli. . . . . Again, there were several instances found in which two young embryos (more advanced than those mentioned in the last paragraph) were developing side by side, no more embryos being present on these prothalli.
>
> The prothallus of *Tmesipteris* is of comparatively large size, and archegonia are present on most parts of it in great numbers, so that the examination of more prothalli in section should show that this form of polyembryony is by no means uncommon. Also I have noticed that the prothallus can continue growing in size after a plantlet has become detached from it. . . . . I have examined a large number of prothalli of each of the following New Zealand species of *Lycopodium*, both externally and in serial sections: *L. cernuum*, *L. laterale*, *L. ramulosum*, *L. Billardieri*, *L. Billardieri gracile*, *L. varium*, *L. volubile*, *L. fastigiatum*, *L. scariosum*. I can give the following facts: The prothalli of the first named three (*L. cernuum*, *L. laterale*, *L. ramulosum*) are comparatively small and short-lived. I have never observed on any of them

more than one young plant or embryo in fertilized archegonium. The prothalli of the next named three (*L. Billardieri, L. Billardieri gracile, L. varium*) are of the much branched epiphytic type, with a comparatively bulky central region on which the sex organs are borne. I have found that many well grown prothalli in all three species have two and even three healthy plantlets. Curiously enough, I have not found a single instance of two or more young embryos or fertilized archegonia existing on the same prothallus, although I have sectioned a large number.

The prothalli of the last named three (*L. volubile, L. fastigiatum, L. scariosum*) are large and deep living, the first two belonging to the *clavatum* type and the third to the *complanatum* type. I have examined a large number of each of these both externally and in serial section. These prothalli are of course all comparatively large in size, and they frequently have two or three developing healthy plantlets. From serial sections I have found on one prothallus of *L. volubile* one young plant and two embryos, and on another four embryos. The prothalli of *L. fastigiatum* supply the most noteworthy instances of polyembryony. One large prothallus showed no less than eleven embryos in different stages of development and three young plantlets, all of these fourteen being healthy. Other prothalli showed from three to five embryos. The archegonia in these two species are nearly exclusively on the liplike prominence which surrounds the top of the prothallus, so that the embryos and plantlets are generally quite close together. In *L. scariosum* I have also found two, three, and four embryos on the one prothallus.

From my own observations I can say that the large growing prothalli of certain *Lycopodium* types (as enumerated above), and also those of *Tmesipteris*, not uncommonly show polyembryony arising from the fertilization of several archegonia. I note, however, that CHAMBERLAIN shows two young plants on a prothallium of *L. laterale* which is of the small-growing form.

It is very evident that *Tmesipteris* and some of the Lycopodiales with large gametophytes may mature several sporophyte plants. Doubtless some kind of embryonic selection is found even among these, as it is highly probable that only a fraction of the zygotes produced attain their full term of embryonic development. Several young sporophytes, however, are usually produced on various parts of the tuberous gametophytes. If these arrested embryos remain healthy looking for a long period, a condition definitely reported for some, this fact would indicate a more primitive condition than that of their complete abortion.

Such a heterosperous form as *Selaginella* is much nearer to the condition from which the seed habit was derived. While some large vigorous gametophytes of *Lycopodium* may frequently give

rise to several sporophytes of some size with or without embryonic selection, these smaller female gametophytes of *Selaginella* that are contained within the megaspore coats do not produce more than one maturing sporophyte. There are, however, a number of archegonia per gametophyte in *Selaginella*, and several figures have been published showing two or three embryos in the same section. One of these is PFEFFER's well known illustration of *S. Mertensii* (fig. 13). When we consider the occasional character of fertilization and some of the difficulties that usually attend this event for a land plant, it is very probable that the fertilization producing these several embryos occurred simultaneously.

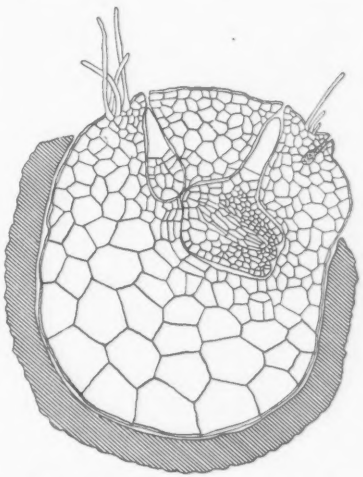


FIG. 13.—Female gametophyte of *Selaginella Mertensii* showing two embryos, ×160; after PFEFFER (33) as modified by GOEBEL (20).

BRUCHMANN (2) states in his monograph on *S. spinulosa* that although several embryos may start to grow, but one comes to maturity. Miss LYON (31), in her paper on *Selaginella*, also shows several instances of polyembryony. The one shown in fig. 14 is given as a possible fertilization of two eggs in one archegonium, or of the fertilization of the ventral canal cell and egg. It is also possible that these embryos were derived from neighboring archegonia, as fig. 15 (drawn to the same scale as fig. 14) would seem to indicate, the embryos having digested the single layer of gametophytic cells that separated their venters. In any event, this shows polyembryony. These embryos are still so small that they are evidently formed from a simultaneous or nearly simultaneous fertilization, and one has already begun to grow a little faster than the other, indicating that embryonic selection is taking place. It appears at least that in some species of *Selaginella* embryonic

selection may play a definite rôle under normal circumstances. In *Isoetes* there are usually several archegonia, so that it is possible for several eggs to be fertilized, at least occasionally, but nothing has been recorded concerning an actual plurality of embryos.

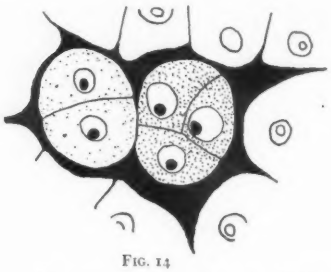


FIG. 14

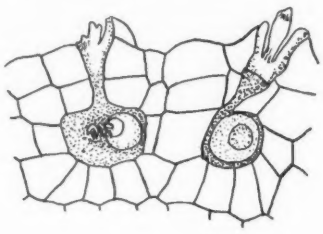


FIG. 15

FIGS. 14, 15.—Embryos of *Selaginella apus* showing polyembryony; fig. 15, fertilization of egg in archegonium beside another zygote; fig. 14 may represent two zygotes of neighboring archegonia such as those in fig. 15 (drawn to same scale) after gametophytic tissue between them was digested away; after LYON (31).

EQUISETALES.—HOFMEISTER (22) definitely states that in *Equisetum arvense* the number of archegonia of a vigorous prothallium is from twenty to thirty. It exceeds, therefore, the number of antheridia of the largest male gametophytes. As a rule more than one egg is fertilized. He counted as many as seven embryos on one such gametophyte. Fig. 16 shows *E. arvense* with two neighboring archegonia containing embryos in competition. In a more recent study by KASHYAP (26) on *E. debile*, the author states that in these vigorous gametophytes the number of archegonia may reach two hundred or more. Although the prothallus may bear only a single sporophyte, eight to ten young sporophytes on a single gametophyte are said to be very common. Under conditions of laboratory culture KASHYAP obtained fifteen or more sporo-

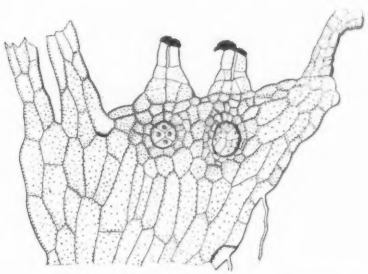


FIG. 16.—Polyembryony in *Equisetum arvense;* after HOFMEISTER (22).

phytes on a single gametophyte (fig. 17). It is difficult to understand how only one or a few eggs could be fertilized where hundreds of archegonia are found, even if they are of successive origin. The result of such a fertilization would produce dozens or at least quite a number of zygotes, a majority of which never develop beyond the stage of only a few cells, and many probably succumb in the struggle for nourishment in the one-celled stage, or before they divide many times. *E. laevigatum* was recently

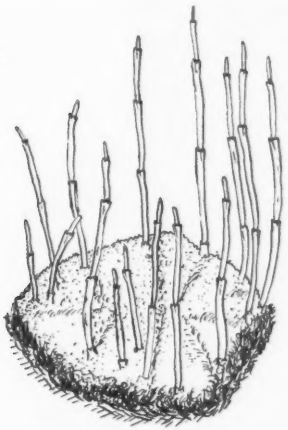


FIG. 17

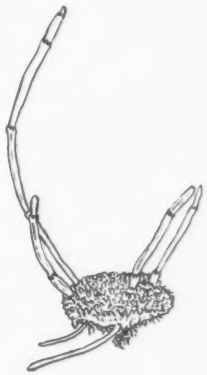


FIG. 18

FIGS. 17, 18.—Fig. 17, gametophyte of *Equisetum debile* giving rise to fifteen sporophytes under laboratory culture, ×1.5; after KASHYAP (**26**); fig. 18, *Equisetum laevigatum*, gametophyte bearing four sporophytes, ×2; after WALKER (**38**).

investigated by Miss WALKER (**38**), and this species likewise has a plurality of young sporophytes, four being shown in one case (fig. 18), and six in another. It is evident that embryonic selection plays a rôle in most, if not all species of *Equisetum*. Plurality of embryos seems to have been found in all carefully investigated species.

OPHIOGLOSSALES.—In his work on the gametophyte of *Botrychium virginianum*, JEFFREY (**25**) states and gives illustrations of the fact that one frequently finds two or more sporophytes on a single prothallium. BRUCHMANN found many very young

embryos in *B. lunularia*, but stated that never more than two could develop on the small gametophyte.

In his studies of *Helminthostachys*, LANG (**27**) found a plurality of young sporophytes, and also found and described many small aborted embryos, whose arrest in various stages of development was due to the supremacy of the larger successful sporophyte. Figs. 19 and 20 are from his figures, made by combining several sections of the series, and show the existence of embryonic selection in *Helminthostachys*. He also gives habitat data which may be taken as evidence that the fertilizations which gave rise to the

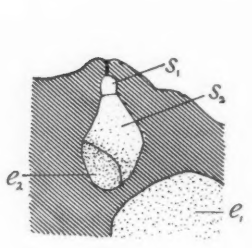


FIG. 19

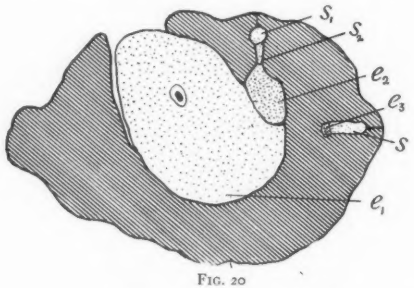


FIG. 20

FIGS. 19, 20.—Fig. 19, arrested embryo (e_2) of *Helminthostachys* beside foot of larger embryo (e_1); after LANG (**27**); fig. 20, two arrested embryos (e_2, e_3) beside larger sporophyte (e_1); s_1, s_2, tiers of suspensor; reconstructed from serial photomicrographs by LANG (**27**).

withered arrested embryos occurred at about the same time as that of the successful sporophyte plant, and that the aborted embryos were starved by the more rapidly developing sporophytes.

Botrychium obliquum has been studied more recently by CAMPBELL (**9**). Something definite concerning the occurrence of embryonic selection may be inferred from this statement in the following passage:

> Unicellular embryos are not uncommon, as several archegonia may be fertilized and begin to form embryos, but the later stages are not so easily found, and it was not possible to secure as complete a series as might have been wished. However, the essential points in the development of the embryos were made out, and there is no question as to the way in which the young sporophytes develop.

Of course embryonic selection would tend to make the later stages scarce, while the arrested unicellular and smaller embryos would be more frequent. Doubtless many of the latter are represented by the aborted embryos studied and mentioned by LANG and CAMPBELL.

LEPTOSPORANGIATE FILICALES.—Embryonic selection is also of common occurrence in many of the leptosporangiate ferns. In a paper on *Osmunda*, CAMPBELL (**7**) makes the statement, speaking of the *O. cinnamomea* gametophyte:

> Frequently more than one archegonium is fertilized as in the Gleicheniaceae (**34**), but as a rule only one embryo develops, although it is not at all uncommon to find several archegonia where the egg has evidently been fertilized, as is shown by its enlargement and investment with a cell wall. Only one case was met with where two larger embryos were present, but one of these was very much in advance of the other, and it is probable that the larger one would have ultimately starved out the other.

RAUWENHOFF (**34**) described the occurrence of several embryos in *Gleichenia* (fig. 22); and in *Vittoria* GOEBEL (**19**) found a similar

FIG. 21

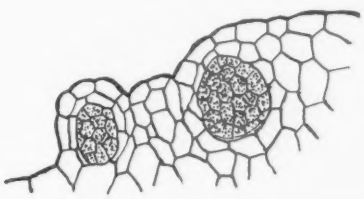


FIG. 22

FIGS. 21, 22.—Fig. 21, gametophyte of *Vittoria* with three embryos (*e*) on various parts of branching thallus: *ar*, archegonia; after GOEBEL (**19**); fig. 22, embryos of *Gleichenia* showing polyembryony; after RAUWENHOFF (**34**).

plurality of sporophytes and embryos. He states that he has no doubt that several sporophytes may come from a single prothallium; at least he frequently noticed several embryos in various parts of the prothallium (fig. 21), also prothallia on which there were still other embryos in addition to the larger sporophyte. GOEBEL states that it is dependent upon circumstances of nourishment whether or not these smaller embryos continue their development.

Among other Filicales this feature of embryonic selection is likewise to be found. MARKLE has prepared many slides of fern embryos in order to supply them for school use, and is therefore able to speak from considerable experience. Most of his material, which includes a variety of leptosporangiate species, was obtained from greenhouses. He has found that by carefully examining his sections in the paraffin ribbons before fixing them to the slides, he has usually been able to make more than one good preparation showing a one-, a two-, or a four-celled embryo on a slide, out of the ribbon obtained from a single large gametophyte. MARKLE states in a letter:

> I do not think I have ever seen more than one embryo on a gametophyte where each had reached the stage with the first leaf evident. I have, however, seen a number of instances where there were at least two or three, possibly four embryos in the two-celled or four-celled stage on one gametophyte. In sectioning material in which the largest embryo was in the stage where the four quadrants have their respective primary organs (foot, root, stem, leaf) well organized, I have seen other small embryos, very evidently suffering from the competition and losing out in the fight with the larger embryos, as was shown by the shrunken appearance of the cells.

Among the fern gametophytes of the preceding discussion, there are quite a few instances in which the several embryos are only those of the somewhat independent or remote archegonial cushions. For example, *Angiopteris evecta* (fig. 23) and *Vittoria* (fig. 21) both have the young sporophytes some distance removed from each other. This condition is found among ferns having large or branching gametophytes, which may have several archegonial groups more or less remote from each other. Among these, as well as among the ferns with large tuberous gametophytes, there is active embryonic selection only when two or more neighboring archegonia are fertilized. Likewise among leptosporangiate ferns the polyembryony has greatest significance when the competing embryos are near each other, as when they are on the same archegonial cushion. This form of embryonic selection, like that of conifers, only rarely produces

FIG. 23.—Gametophyte of *Angiopteris evecta* bearing two young sporophytes; after FARMER (18).

more than one sporophyte from a single gametophyte. This condition of polyembryony, which was casually mentioned by several students of pteridophytes, is well illustrated by fig. 24, which is a species of *Aspidium*, probably *A. Thelypteris*, collected in its natural habitat. While the larger embryo (fig. 24 *A*, e_1) has become multicellular, having approximately 25–30 cells, the second one (fig. 24 *B*, e_2), found on the remote side of the archegonial cushion, has remained unicellular, but has enlarged considerably.

Fig. 25 is from a gametophyte collected on the benches of a greenhouse whose species could not be determined, but could have been one of a half-dozen leptosporangiate species growing near by.

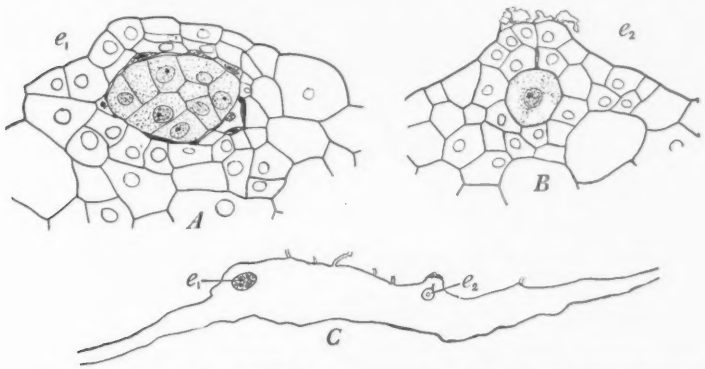


FIG. 24.—Embryonic selection in *Aspidium: A*, enlarged view of larger embryo of *C*, surrounded by calyptra; *B*, smaller unicellular embryo shown in *C* on same gametophyte, less than 1 mm. distant; (*C* reconstructed from several serial sections); *A* and *B*×200, *C*×42.

This is included here because it seems to show a stage slightly later than that of fig. 24, and indicates the fate of the smaller embryo of the latter. It is especially interesting to note the shrunken and starved appearance of the embryo in fig. 25 *B*. That the larger embryo starves the smaller is a very natural explanation; this is a factor which is very certain to be involved, but it is also possible that the excretions of one embryo tend to inhibit the development of the others. In ferns having large vigorous gametophytes with many archegonia, if the aborted embryos are not too quickly starved they should be subject to recall experimentally,

by any measures which would tend to prevent this embryonic competition.

From a careful examination of a number of species of leptosporangiate ferns, it is clear that there is a considerable period of enlargement of the egg following fertilization, before the zygote divides. It is obvious that the decisive part of the embryonic selection may transpire during this early period, so that most if not all of the zygotes are eliminated before they have undergone cleavage.

A few examples may be given showing the amount of increase in volume during the first stages of the embryogeny, calculated

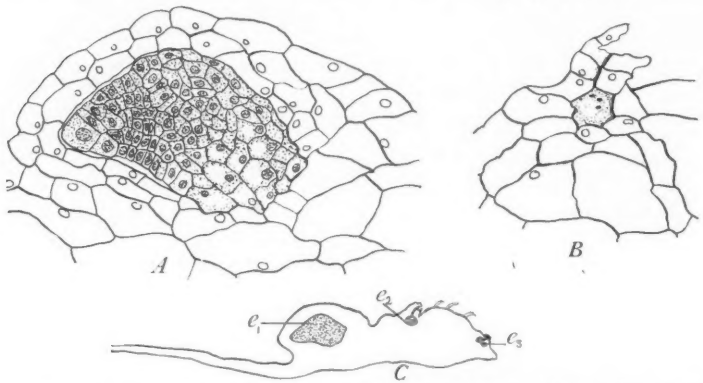


FIG. 25.—Fern gametophyte with several embryos in competition, representing slightly later stage than fig. 24; *A* is e_1 enlarged; *B* is e_2 (second unicellular embryo) with disintegrating nucleus, collapsing as it is aborted through embryonic competition; e_3 appears to be a third collapsed unicellular embryo (?); *A* and *B*×200, *C*×42.

from micrometer measurements. The average of several fertilized eggs in *Osmunda cinnamomea* measured 6000 cubic microns, while the zygote of the same species after only one cleavage measured 19,000 cubic microns. In *Adiantum* the diameter of the egg measured 17,000 cubic microns, while the two-celled zygote measured 65,000 cubic microns. In a species of *Pteris* the fertilized egg measured 33,000 cubic microns or less, while the two-celled zygote exceeded 195,000 cubic microns. Thus it appears that among leptosporangiate ferns there is a definite enlargement of the zygote of from 200 to 500 per cent during the first cleavages.

In eusporangiate ferns with well developed suspensors it is very much greater; hence the evidence to be found in the eliminated zygotes of this more active form of embryonic selection in ferns is not conspicuous. It occurs in the earliest stages, leaves only very small aborted embryos, and it is probably for this reason that embryonic selection has usually been overlooked as a normal process of the life cycle.

While many of the living ferns probably do not possess embryonic selection, at least as a very striking or prominent feature, practically all of them show good evidence of a derivation from forms possessing it; of having passed through this condition historically. During Paleozoic time, when pteridophytes constituted the dominant vegetation, embryonic selection was probably the prevailing condition. Even the environmental forms of competition were much more keen, as our vast coal deposits would indicate. There is little doubt that the early seed plants which were derived from these ferns retained embryonic selection, as it is a feature which has persisted until today in gymnosperms generally. The simple polyembryony of gymnosperms is therefore of fern origin.

Suppression of embryonic selection in ferns.—There are some pteridophytes among which there is a more or less complete elimination of embryonic selection. Such a highly specialized form as *Marsilia* presents a special variation in this direction, since only one archegonium is produced on the female gametophyte. Obviously there is no selection between two or more zygotes on the same prothallium. It appears that in *Marsilia* and other pteridophyte forms having only one archegonium, we have examples of the elimination of the embryonic selection, an advanced condition, doubtless the result of specialization. Leptosporangiate ferns, whose gametophytes are sometimes very much reduced in size, may perhaps provide additional interesting examples of the complete elimination of embryonic selection. Should this selection occur between archegonia, or between the eggs of neighboring archegonia during their development, it could certainly not be classified as embryonic selection; it may perhaps be designated as gametic selection, or in some other category of developmental selection.

It is needless to point out that morphological investigators have been concerned with only those stages in the development of the individual sporophyte or embryo which constitute the more important links of the life cycle, only occasionally illustrating or noting the occurrence of several embryos on one gametophyte. Sometimes an investigator illustrates a plurality of embryos without further comment, and LANG in his work on *Helminthostachys* reported a number of embryos only because he made use of the arrested embryos of one gametophyte in describing some of the missing stages of embryonic development.

STEPS IN EVOLUTION OF EMBRYONIC SELECTION AMONG PTERIDOPHYTES.—If definite steps in the evolution of embryonic selection among pteridophytes are recognizable, these may serve as a rough measure of their phylogenetic position, at least of the relative position within each of the several well recognized groups. It would seem that at least the following stages or steps in the evolution of embryonic selection may be recognized.

1. Many sporophytes are found on one vigorous gametophyte, a large portion of which reach maturity. Selection may finally occur under conditions of crowding in early or later stages, but this elimination occurs largely in the environment, and must then be recognized as natural selection, as, for example, *Tmesipteris* and *Lycopodium* with large vigorous gametophytes.

2. A few sporophytes appear above the soil or break through the tissues of the gametophyte, but a selection occurs among a much larger number during their embryonic stages; arrested embryos remain turgid for a considerable period, as for example *Lycopodium*.

3. One or only very few sporophytes break through tissues of gametophytes, but a selection occurs among a large number in their embryonic stages; arrested embryos are soon aborted and not easily recognized, as, for example, *Equisetum*, *Helminthostachys*, and *Botrychium*.

4. Normally only one sporeling sporophyte is produced, but several archegonia are fertilized, and selection between zygotes occurs in early embryonic stages; arrested embryos are usually soon aborted and not easily recognized, as, for example, *Selaginella*, *Osmunda*, *Aspidium*, etc.

5. Gametophytes are so reduced in size that only one archegonium is produced, making selection between embryos of separate fertilization impossible, as, for example, *Marsilia* and *Pilularia*.

Selection between gametes

Among all of these pteridophytes another form of developmental selection may be recognized. Doubtless a selection occurs among the male gametes as they swim to the archegonia. That the archegonia attract the sperms chemotropically has long been known. The gametic selection is therefore a measure of their response to this stimulus. While it may be largely a matter of chance which of the many sperms that reach the archegonium and swarm about its neck actually reach the egg to effect the fertilization, there can be no doubt that the less active sperms or those otherwise defective would be eliminated in the race to reach the egg. If only the most vigorous sperms take part in fertilization, and there seems to be very good ground for this, certainly a form of gametic selection is to be recognized. It may be noted in passing that with its return to aquatic life, the natural sphere of swimming sperms, *Marsilia* has exchanged one form of developmental selection for another. Embryonic selection was made impossible and lost through reduction of archegonia, but gametic selection was doubtless facilitated when this fern returned to the aquatic habit. Gametic selection is not a new suggestion, having been suggested by THOMSON (**36**) for animals, and it is probably in part along this line that the principle of developmental selection may be found to apply somewhat generally to the animal kingdom.

It must be remembered that no reduction divisions occur in the formation of sperms in ferns, nor is any special form of cell division known or recognized here which might bring about genetic changes. In animals the formation of sperms is accompanied by a chromatin reduction both equational and differential, a condition shared by some algae, notably *Fucus*.

OTHER FORMS OF DEVELOPMENTAL SELECTION.—There are still other forms of developmental selection which must be taken into account. One of these is illustrated by the selection which takes place at a certain stage of development in *Selaginella* between the

megaspore mother cells, a large number of which begin to appear only to degenerate when one of them is selected and enlarges to form the single tetrad of megaspores. This selection is neither embryonic, gametic, nor gametophytic, but belongs to a distinct category, somewhat similar to some other types of developmental selection which will be discussed later.

Developmental selection among spermatophytes

EMBRYONIC SELECTION.—Most striking of the forms of developmental selection of seed plants is the embryonic selection illustrated by the polyembryony of gymnosperms, which has already been described in a general way. The several embryos originating from the fertilized eggs engage in a competition in which the most vigorous individual is always the winner. Not only must the embryo rapidly become massive and multicellular, but it must also produce a stiffer and more vigorous suspensor, one which keeps the successful embryo in the commanding position. The winner is usually the foremost of the group of embryos, where the embryonal tubes of the elongating secondary suspensor are able to push the other competitors back, away from the most favorable position. This applies whether cleavage polyembryony occurs or not. The mature conifer seed has a single large embryo, but the remains of some of the other embryos participating in the competition can usually be found, crushed against the archegonial end of the embryonal cavity within the gametophyte (endosperm) by the suspensor or radical end of the successful embryo.

GAMETOPHYTIC SELECTION.—Another type of developmental selection is gametophytic selection. This is not intended to apply to the form of natural selection occurring between independent gametophytes, as those of liverworts or ferns, in the external environment, but rather to a plurality of male or female gametophytes which are dependent on a sporophyte, as they are in seed plants. It is well illustrated by the pollen tubes of a pine or other conifer whose competition predetermines in a measure which of the several archegonia shall first be fertilized. Although fertilization in conifers is almost simultaneous even in the various cones of the same tree, a fact first pointed out by HOFMEISTER (21), this

event does not usually occur absolutely simultaneously even in a single ovule. Some embryos are usually produced a little earlier than the others, and have a slight advantage, although this difference may represent only a few hours or minutes. The competition is continued between the several embryos in the ensuing embryonic selection, which becomes truly a struggle for existence, so that the resulting seed has only one embryo. Here in gymnosperms the developmental selection process is a sort of relay race between one pollen tube plus its embryo, and other pollen tubes plus their

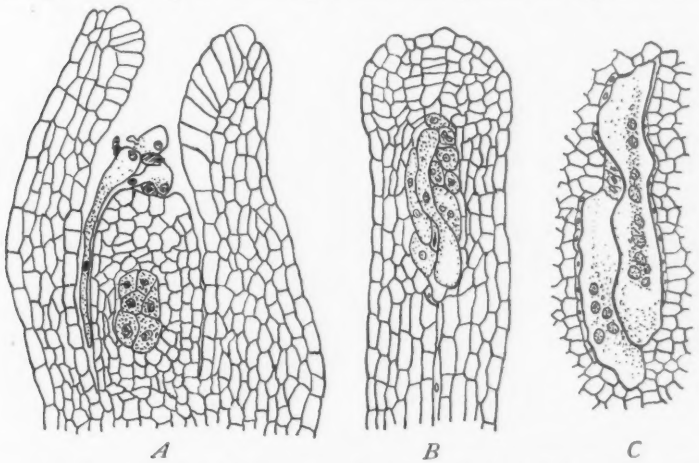


FIG. 26.—Sections of ovules of *Sequoia sempervirens*, showing selection between female gametophytes: *A*, ovule with six megaspore mother cells; *B*, numerous germinating megaspores; *C*, older stage showing two young gametophytes whose competition has persisted into multinuclear stage; after LAWSON (**28**).

embryos. The embryo of a gymnosperm seed is therefore the survival of a rapidly developing pollen tube combined with a very vigorous embryo.

Among conifers the male gametophytes are not the only individuals taking part in this competition. Female gametophytes may also undergo competition under normal conditions in some species. DUPLER (**16**), in his work on *Taxus canadensis*, showed that the existence of several female gametophytes arising from as many megaspores is quite the normal condition. LAWSON (**28**) found a similar situation in *Sequoia* (fig. 26), and apparently also

in *Cryptomeria* (29). The work of LOTSY (30) on *Gnetum* indicates the same thing, and occasionally slight evidences of this are found in *Pinus* and other conifers.

Even angiosperms sometimes have this competition between female gametophytes, which seems to occur as a functional form of developmental selection. This is especially true of some of the lower Archichlamydeae and Monocotyledons. *Casuarina* (37) has been reported to have as high as twenty or more megaspores, of which several enlarge considerably, but only one is functional. *Alchemilla* (20) has been observed with five or six ripe megaspores;

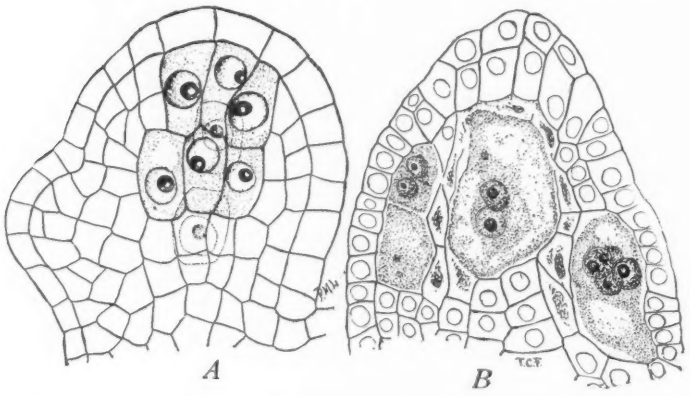


FIG. 27.—Selection between female gametophytes in *Ranunculus septentrionalis: A*, section of nucellus showing eight-celled archesporium; *B*, later stage showing several female gametophytes and aborting megaspores in early stages of development, ×400; after COULTER (12); cut lent by D. Appleton Company.

in *Arisaema* (8) something similar has been reported; and in *Ranunculus* COULTER (12) found as many as eight archesporial cells and three embryo sacs within the same ovule (fig. 27). Numerous additional examples are on record. GOEBEL (20), in discussing the many gametophytes of *Casuarina*, suggests that "biologically this repeats the case of the embryos of the Abietineae, where, of the many embryos which arise from one egg, only one develops." His interpretation of the significance of polyembryony in conifers is treated as a process of correlation, where he compares it to the

correlative "struggle" between vegetative parts, or flowers in a crowded inflorescence. It is evident that he recognized a significant similarity in these forms of developmental selection, although apparently he did not anticipate the significance of these facts in relation to the selective mechanism of evolution.

A very unique form of developmental selection is represented in *Welwitschia mirabilis* described by PEARSON (**32**). The female gametophyte gives rise to a number of nuclei, potentially eggs, which develop prothallial tubes that grow up into the nucellar tissue. When such a prothallial tube comes in contact with a pollen tube, fertilization takes place. The embryos may be found growing down through these prothallial tubes into the female gametophyte tissue. Although we have polyembryony, the selection is probably in part predetermined by priority of fertilization, which depends upon the pollen tubes and the prothallial tubes of the female gametophyte. Apparently the selection resolves itself, in part at least, into a competition between eggs, or prothallial tubes containing eggs, a form of selection between female gametes which is very rare in plants.

Megaspore tetrad formation and the abortion of the megaspores in angiosperms might suggest itself as a form of developmental selection, but the selection in this case seems to be largely one of position. It is not any megaspore of the group in a linear tetrad that may give rise to the embryo sac, but almost always the innermost of the four. This selection is not dependent on the physiological success of the megaspore, but is morphologically fixed, and therefore not properly included among processes of developmental selection.

Among angiosperms the selection between male gametophytes or pollen tubes represents the most important developmental selection machinery. In the pistil of the ordinary flower an excessive number of pollen grains may germinate on the stigmatic surface, but usually only a limited number of these can function in fertilizing the eggs within the ovules. Only one pollen tube is necessary to fertilize the single ovule in the pistil of maize, yet hundreds may fall on each stigma and germinate, producing pollen tubes of varying lengths. Fig. 28 represents the pistil of an

angiosperm, illustrating the important mechanism of this gametophytic selection. A mature cotton boll contains an average of from 30 to 40 seeds, yet hundreds of pollen grains may germinate on the stigma of the pistil. EAST (**17**) has determined by actual count that there are usually 1200 to 2000 pollen tubes in a single pistil in tobacco, sufficient to fertilize from four to six times the number of ovules in the ovary.

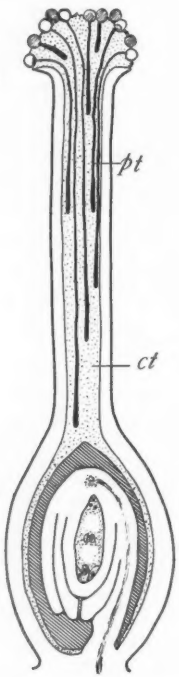


FIG. 28.—Diagram of pistil of angiosperm having one ovule, showing pollen tubes (*pt*) penetrating conducting tissue (*ct*) of style, engaged in competition to reach ovule, resulting in gametophytic selection.

Recent genetical studies have shown that this gametophytic selection in angiosperms represented by the pollen tubes has a significance of the first importance. For example, CORRENS (**14, 15**) has shown that in *Melandrium* there is a selection between the male gametophytes in their race to the ovules, that the female-producing pollen tubes are on an average decidedly faster in their growth than those carrying the factors which produce males. By applying much pollen so as to crowd the pollen tubes, he was able to increase the pollen tube competition in favor of the production of more females, and by sparse pollination he was able to stay this competition somewhat, resulting in the production of more males than under normal conditions. This may be taken as definite experimental proof of gametophytic selection in angiosperms.

Experimental studies of pollen tube competition in angiosperms have been in progress for some time by the writer, and will be published in separate papers. It may be stated here that gametophytic selection, as it affects the evolution of angiosperms, is a subject open to experimental study. It is already apparent that the genetic result of this gametophytic selection is a matter of the first importance if it relates to some of the heritable characters,

especially in plants with long styles. While this pollen tube selection in angiosperms is morphologically very different from the embryonic selection in gymnosperms, it is physiologically a very similar process. In the case of the embryonic selection the embryo sporophytes are digesting their way and pushing forward into gametophytic tissue, while pollen tubes represent gametophytic structures penetrating sporophytic tissue by what appears to be a very similar method.

An important difference, however, between the gymnosperm embryonic selection and the pollen tube competition of angiosperms should be noted. The embryos concerned in the competition are diploid individuals, while the pollen tube and other forms of gametophytic competition take place between haploid individuals. The recent work of geneticists shows that lethal factors may be present in one member of a pair of chromosomes apparently without serious consequences, as long as the same lethal is not present at the same time in both chromosomes. Factors lethal to the gametophyte could not be protected in this way by a homologous chromosome.

INTEROVULAR AND INTERFLORAL SELECTION.—Another form of selection, belonging more or less completely to the categories of developmental selection, is that occurring in angiosperms between the ovules within the same ovary. In species of *Quercus*, for example, there are six ovules within the ovary, although normally only one ovule with one embryo is found developed in the acorn which matures from this ovary. In *Fraxinus* and in the olive there are two ovules in each pistil, yet only a single seed with one embryo is matured. There are numerous similar instances in the plant kingdom, and whether the elimination of the unsuccessful ovules in such cases occurs as early as the stage when the megaspores or female gametophytes are developing, or only among the ovules containing zygotes after fertilization has taken place, remains to be determined. According to the published accounts of the morphology, the latter is probably what happens in *Quercus*. The selection between reproductive organs during early vegetational stages, as that previously described for *Selaginella*, should be included in this category of developmental selection.

Interfloral selection is a form that may occur between the individual florets of a crowded inflorescence, such as the head of Compositae, the spike of maize, or the umbelled cluster of flowers in *Asclepias*. It is to be referred back to a struggle between the embryos contained in the different flowers, but since this competition is indirect, it is not usually very decisive. The process is less secluded from environmental influences, and takes on a form somewhat similar to the selection between vegetative branches described later. Interfloral selection is of little consequence in evolution as a form of developmental selection, except probably in rare instances.

VEGETATIVE FORMS OF DEVELOPMENTAL SELECTION.—Thus far the reproductional phases of developmental selection have been the chief concern. These reproductional types of selection are by far the most important, since through sexual reproduction new zygotes combining diverse hereditary strains come into existence. The developmental selection which takes place during reproduction, therefore, is a kind which may occur between different phenotypes, and produces results that are genetically very significant.

That there are also vegetative forms of this developmental selection process should not be overlooked, but this vegetative selection remains within the same genotype, unless a vegetative mutation occurs. For example, the branches of a tree are in a state of competition for light and favorable exposure. A struggle for development was suggested long ago by MÜLLER, who pointed out that there are many times more buds on every twig of a tree than can possibly develop into branches. While external circumstances of exposure may largely determine the result of this selection, the merits of the individual buds and their branches are also responsible in part for the result. If bud mutations occur, this vegetative selection determines at once whether they shall survive to reproduce themselves later or be eliminated. The principle is very largely the same for any form of vegetative selection, whether in gametophyte or sporophyte, by the dichotomy of thallus, aerial branches, stolons, tubers, rhizomes, or roots. When practiced for the purpose of obtaining vegetative mutation, bud selection has been called clonal selection. Of course, it is evident that this selection

does not belong rigidly to the categories of developmental selection, for it is subject very largely to external environmental conditions. It is not entirely in the external environment, however, since internal physiological correlations are concerned. It is clearly a type of selection standing between natural selection and developmental selection, as previously indicated.

Summary

Embryonic selection and gametic selection represent the important forms of developmental selection found among pteridophytes. Among gymnosperms the gametophytes plus the embryo usually take part in this competition, and among angiosperms it is typically a competition between male gametophytes represented by the pollen tubes. The important reproductive developmental process is merely shifted to an earlier stage of the life cycle in passing from ferns to angiosperms, where vegetative and other less important forms of developmental selection are also found.

Developmental selection in relation to natural selection and mutation

It is very evident that a good selective process, whether developmental, artificial, or natural selection is meant, should meet at least the following four requirements. It should (1) start the competition simultaneously, (2) take place under uniform conditions, (3) measure comparable merit, and (4) rigidly eliminate the great majority that fall below the standard.

First it should launch the individuals who participate into the competition with an even start. This is a primary requirement in any kind of a performance race. Well fitted is the process of fertilization to launch the competition of embryos, as this is a relatively sudden event, one which may happen only occasionally, and may be simultaneous in producing several or a large number of zygotes. Similarly the shedding of sperms from an antheridium provides an even start in the competition of sperms, with a definite goal to be reached through their activities. Pollination, especially when the pollen is transferred in masses or clumps by insects, is another more or less sudden event, which launches the competition

of pollen tubes. All of these events which start the various processes of developmental selection are superior or at least equal to the equivalent initiatory processes of natural or artificial selection. Among the latter seed germination which, while it gives a fairly even start to many of the competitors, is a more variable process, easily modified by soil conditions or delayed and unequal germination; it is slower and it is not usually as efficiently simultaneous as pollination or fertilization.

Selection between vegetative parts is also initiated by the awakening of the buds in the spring, a process which may be more or less simultaneous and comparable with seed germination under the most favorable conditions. Birth and the hatching of eggs in animals is a process well fitted to initiate the competition of natural selection. It is apparent that the processes of fertilization, pollination, and the liberation of sperms are all very superior means of beginning a selective process, and that there are only a limited number of these events in the life cycle of an individual.

The second requirement, that the competition should take place under uniform conditions, is one in which developmental selection excels, while natural selection is very inefficient. Under the conditions of isolation in pure culture in artificial selection, the environmental conditions are made very uniform, but even here the conditions are not as isolated and insulated as they are within the ovule of the pine seed, or within the tissues of the stigma and style, where pollen tubes must carry on their competition. On the other hand, the external environment where natural selection occurs is exceedingly complex and diverse.

The third requirement, that selection should measure equal merits, is also one in which natural selection falls far short of providing the best possible mechanism. In the external environment not all of the competing individuals which are "saved" are required to go through exactly the same performance, at the same time, in the same place, and under the same conditions. So many and varied are the factors that might be used to determine survival, and so different are the responses of plants that might be made to them in obtaining survival, that the capacity for an equal performance of the same task under similar conditions is not measured,

but rather a general indefinite all round fitness. It has already been shown, however, how developmental selection sets the same task for all the competitors. Likewise artificial selection sets a uniform standard of excellence of performance for all participants, but a standard which is limited by the powers of discrimination of the breeder.

By providing a very uniform medium for the competition of embryos, pollen tubes, etc., and by forcing rigid elimination, developmental selection precludes indiscriminate survival. The surviving plant may owe its existence to the performance record of its parent pollen tube, or it may owe its existence to its own performance during embryonic development; but in any event, there are very definite measures of some kind of excellence to be lived up to on a competitive basis. The surviving individuals constitute a class, selected for their superiority among several, among dozens, or even among hundreds of other individuals which were destroyed in this competition. Developmental selection, therefore, is not open to this objection which has been urged against natural selection.

In the fourth requirement, developmental selection again excels, while natural selection is only feebly effective, for the defeated individuals are rigidly eliminated in developmental selection. The losers in the environmental competition are not always destroyed from reproduction; their progeny may only be diminished somewhat. Artificial selection also meets this requirement fully.

In connection with these special features of developmental selection, it is interesting to consider some of the objections which have been raised against natural selection. One of these concerns the chances of death, which have been ably discussed by several evolutionists, who point out that the destruction of individuals is very indiscriminate, that the fittest do not always survive, for many of them are destroyed. Likewise, the least fit do not always perish. Thus it has been urged that there is little evidence that natural selection actually selects any specific class of individuals in preference to others. In fact, so complex is the environment in which natural selection must sort out the superior, that accident and chance really play a major rôle.

Another of the objections which have been urged against natural selection is that this theory rests altogether too largely on an unwarranted analogy with the process of artificial selection, although this supposed analogy was a very convincing argument in the hands of DARWIN. This analysis shows how natural selection is weak in at least three out of four of the requirements in which developmental selection excels, and how a much closer parallel or analogy may be drawn between developmental selection and artificial selection.

That there are many details which do not permit a close parallel between natural and artificial selection may further be illustrated. For example, the breeder in practicing mass selection, plants a large number of seeds in a uniform soil, and seeks to eliminate all other environmental differences wherever possible. Pure breeding in isolated cultures is possible, and at some definite stage when the seedlings come up, or as they mature, they may be measured and selected by very nearly the same standard of size and growth vigor, color, size of fruit, disease resistance, etc. Natural selection must necessarily be a much less methodical process. In nature, survival must be determined on the basis of a total or all round fitness. Very often this survival is purely fortuitous. Naturally disseminated seeds are less likely to germinate simultaneously in a uniform environment than planted seeds in a cultivated soil. If seedlings do not get an even start, are not growing in a uniform environment, are not measured up to the same standard, and the unselected are not always destroyed, survival by chance plays a very important rôle, and their apparent competition cannot be one of the greatest consequence. This has been urged as a very serious objection to natural selection even as a highly efficient selective mechanism, aside from the question of its power in originating species. Obviously the mechanism of developmental selection is much better fitted to bring about a competitive form of selection. It may be considered more efficient even than artificial selection, where uniformity of environment is only approximate, and the standards of selection depend upon the discriminative powers of the breeder. Finally, developmental selection makes possible a very early decision, which is doubtless a most valuable

form of biological economy, another feature in which developmental selection excels.

A further objection which has been made to natural selection has to do with the difficulties that are involved in explaining how the first steps in any given variation may be of selective value. How can natural selection influence a structure whose advantage is to be reached only at some future time, after the results of the selection are achieved? This is asking natural selection to pass on a prophecy. Developmental selection, however, is a form of selection which can act on some very small quantitative characters. For example, by playing on such features as minute differences in suspensor length and rate of growth in gymnosperms, or a rapid pollen tube penetration in seed plants, developmental selection brings about a positive selection for minute differences in these particular characters. It may be asked how this selection for embryonic or pollen tube vigor could in any way affect a selection for other characters. The answer is found in linkage of characters. A sporophyte character of the mature plant must be linked with the factors producing either vigorous suspensors and embryos, or vigorous pollen tubes in the gametophyte stage.

CORRENS found just such a case in *Melandrium*, which was referred to previously, in which the female-determining pollen tube has the quality of growing slightly faster than the male-determining pollen tube. Judging from his account, this linkage is probably not an exceedingly close one, as he states that there are some male-determining pollen tubes that grow faster than the slowest female-determining pollen tubes. Such a condition may be due to the well known crossing-over phenomenon associated with linkage, between the sex factor and the gene or genes producing rapid pollen tube growth. If factors affecting the rate of pollen tube growth should become linked with quantitative factors, which are usually multiple factors affecting the size of an organ or part, it is easy to understand how developmental selection can play on them indirectly through their linkages. An accumulation of the effect of this selection would account for the building of a new structure. Of course natural selection can also play on such a character or structure when it or a factor linked with it has become

distinctly useful or harmful to the organism, but not before the structure is present.

DARWIN recognized such linkage, calling it correlative variability. He accounted for many useless characters by assuming their linkage with other factors which are sufficiently useful to have a survival value. It has already been shown, however, that there is great difficulty in definitely pointing out that certain characters are actually acted upon in the environment by natural selection on a consistent basis. In developmental selection there is no doubt about a definite selection taking place, and the experimental evidence that certain adult sporophyte characters are linked with the gametophytic or embryonic factors having definite values in selection is also available.

This will also explain the production of overdeveloped structures, or those having no advantage to the organism. Suppose the factors determining the length or shape of the spines in the ends of some pine cone scales, or other useless details of the plant, should become linked with the genes producing either the most vigorous pollen tubes or embryos, there is no doubt that the selection for the pollen tubes or the embryos would result in the selection of the other characters in the same linkage. The spines would then be selected in or out, as the case may be, by developmental selection. Details of variations or mutations, be they ever so small, may either tend to disappear or become fixed or overdeveloped, as they are affected by this internal selective mechanism. Such phenomena of determinate variations and evolution in definite directions have long been recognized, and have been attributed to a principle called orthogenesis. The mechanism of developmental selection described here looks very promising as a means of accounting for many or thogenetic phenomena.

It is probably needless to point out that many characters would not be linked with these factors involved in developmental selection at all. These would remain unaffected by the developmental selection processes. Among these most of the heritable characters that have been studied by the Neo-Mendelians are to be found. The genes of these characters segregate independently of the factors affected by this selective mechanism, and yield Mendelian ratios in the expected proportions. Among those which should probably be

classed as the ones affected by some form of developmental selection are many of the lethal factors that have been described in some plants. It has been shown by LITTLE that some kind of embryonic selection is responsible for the non-appearance of homozygous yellow mice whose inheritance was studied earlier by CUENOT. No attempt will be made to discuss here the lethal factors of *Drosophila* in this connection, but doubtless many things whose inheritance would follow the lethal type, could be caused by a kind of embryonic selection mechanism.

The origin of the variations or mutations is another problem. Chromosomal phenomena, such as gene mutations, the chromosomal mutation of non-disjunction, etc., are probably able to account for the actual origin of many variations. Similarly bud mutations and other heritable vegetative variations would also be accounted for by some type of nuclear or intracellular phenomena. They would be played upon by developmental selection even before their outward manifestations are recognized. These mutations may also be acted upon by natural selection if they possess some very marked advantage or disadvantage. It is in this connection that developmental selection has a very definite rôle in the origin and heritability of some mutations. Only those variations which are not affected by developmental selection could reappear regularly or give consistent Mendelian results. The outcome of developmental selection may be so decisively against a mutation that it may seldom recur. On the other hand, if the mutation is closely linked with a factor greatly favored by developmental selection, it may reappear as if fixed, even though the pollen produced is heterozygous for it. Thus we can understand how even the discovery and recognition of the mutations themselves depend upon whether they reappear in the next life cycle, and thus pass the censorship of the developmental selection machinery. Developmental selection is doubtless responsible for the recognizability of some variations as mutations, but we have no evidence that it could be held responsible for the chromosomal phenomena themselves.

Summary

1. The process of developmental selection is a normal event or succession of events in the life cycle of vascular plants, where it

assumes various forms, being represented chiefly by embryonic selection, gametophytic selection, and gametic selection.

2. Developmental selection differs materially from natural selection, germinal selection, the intraselection of ROUX, as well as the other selection theories.

3. Records of conspicuous cases of polyembryony in ferns are brought together. Original studies are added, constituting definite evidence that a selective plurality of embryos may normally exist even in the leptosporangiate ferns. Nearly all living ferns seem to have embryonic selection, or show evidence of having passed through a stage in which embryonic selection was the normal condition. The embryonic selection represented by the polyembryony of gymnosperms was derived from an embryonic selection habit in their fern ancestors.

4. Developmental selection in gymnosperms and angiosperms is not only represented by a selection among embryos, but also by a selection between female gametophytes and the male gametophytes represented by the pollen tubes.

5. A form of selection intermediate between natural selection and developmental selection may be recognized in the competition between buds and branches of a sporophyte or a branching thallus.

6. Developmental selection is a process which brings into play a definite internal competition between embryonic diploid individuals, as well as between the haploid sperms of fern plants, and the haploid male and female gametophytes of gymnosperms and angiosperms. On the other hand, natural selection usually acts on the diploid generation in these plant groups, or on the haploid fern gametophytes, where selection may take place in the external environment.

7. The discussion seeks to show why the process of developmental selection is not open to the more serious objections which have been urged against natural selection, and on what basis it equals or excels the latter as an effective selective process.

8. The discussion also shows how developmental selection may account for some of the phenomena of orthogenesis on a mechanical basis.

9. Developmental selection is not responsible for the origin of the chromosomal or other intracellular phenomena involved in

mutation, but it is a powerful mechanism whose censorship may determine whether or not any particular intracellular phenomena causing mutation may complete the life cycle to be heritable, and therefore recognizable as a mutation.

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BIOCHEMISTRY OF PLANT DISEASES

III. EFFECT OF SCLEROTINIA CINEREA ON PLUMS[1]

J. J. WILLAMAN AND W. M. SANDSTROM

(WITH SEVEN FIGURES)

From the viewpoint that a fungus attacks a host plant, not to destroy it, but to gain a livelihood, it becomes of interest in the study of the chemistry of resistance to discover why a fungus can parasitize some varieties of a host and not others. There may be several bases for this difference: (1) the structure of the resistant host may offer mechanical difficulties to the entrance of the parasite; (2) the host may contain or produce repellent substances, such as tannins, acids, antienzymes, and antibodies; or (3) the host may fail to furnish the proper kinds and amounts of nutrients for the normal development of the fungus.

Each of these possibilities has received some attention at the hands of investigators; but the two latter, constituting what may be called the biochemical basis of resistance and susceptibility, have received the least. It was decided, therefore, to attack the problem of resistance and susceptibility in plants from the standpoint of the nutrition of the parasite, using the brown rot organism of stone fruits, *Sclerotinia cinerea*, as the experimental organism. The first paper in this series dealt with the vitamine requirement of the fungus (**52**), the second with its relations to the pectic substances of the host (**53**); the present paper deals with the composition of certain varieties of plums, and the changes in composition brought about during the process of rotting by the fungus.

Previous work

COOK and TAUBENHAUS (**16, 17**) found that a great many fungi are very sensitive to tannin, and they believed that this could be a limiting factor in their ability to attack certain plants. In general, parasites are more sensitive to tannin than saprophytes.

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They demonstrated an oxidizing enzyme that produces tannin from gallic acid, and found that the abundance of this enzyme in fruits is correlated with their resistant properties. BASSETT and THOMPSON (3) studied this enzyme still further. KNUDSON (37) found that *Aspergillus* and *Penicillium* can utilize tannin as a source of carbon, by means of the enzyme tannase. That genera and even species within a genus vary greatly in their sensitiveness to tannin was shown by COOK and WILSON (18) in studying the chestnut blight. In the case of *Sclerotinia*, VALLEAU (46) failed to find any correlation between tannin content and resistance in plum varieties.

In recent years considerable attention has been given by investigators to the relation between the H-ion concentration of the soil and that of the plant, and between the latter and its resistance properties. In 1912 COMES (15) announced that the wheats that were more resistant to rust had more acid saps, and that fertilizers which would increase the acidity of the sap would convey added immunity to the plant. Although others have since failed to corroborate these statements, positive correlations have been established between the acidity of the soil and the occurrence of potato scab (23, 24), between the acidity of grape saps and their resistance to disease (2), between the H-ion concentration of soil and that of the plant juice (14, 45), and between spinach mosaic and the PH value (27). On the other hand, a lack of correlation between the acidity of the host and its resistance properties has been found in the case of potatoes toward *Pythium debaryanum* (32), *Phytophthora infestans* (35), and *Chrysophlyctis endobiotica* (50). WAGNER (47, 48) noticed that certain plants increased their acidity when infected with bacterial pathogens. The acidity returned to normal after a brief period, unless the plant were unable to withstand the attack, in which case there took place a sudden fall in acidity much below normal, the death of the tissue, and then a post mortem rise in acidity. The relation of H-ion concentration to the metabolism of fungi and bacteria has received some attention (25, 38, 49). SCHMIDT and HOAGLAND (41) give an extensive bibliography on the relation of bacteria to the reaction of the medium. In general the fungi have been found to be less sensitive than bacteria to the reaction of the medium, and hence fewer

instances are known where the reaction of the sap is a controlling factor in a fungus disease. In the case of *Sclerotinia cinerea* no measurements have been made of its relation to the P_H value of the medium, except some rough titrations made by COOLEY (**19**) on cherry juice, which showed that this fungus can grow and sporulate through a considerable range of acidity and alkalinity.

Many investigators have sought for the mechanism by which fungi penetrate through the tissues of the host plant. Without going into the voluminous literature on this subject, it may be said that several different ways have been found: (1) by mechanical pressure, (2) by enzymes which dissolve either the cell walls or the middle lamellae (**12, 13, 19, 46, 53, 54**), and (3) by toxic substances other than enzymes, especially oxalic acid (**6, 7, 9, 12, 13, 19, 42, 46, 53, 54**). COOLEY found oxalic acid produced by *S. cinerea* in small amounts, and VALLEAU demonstrated that solutions of this acid would disintegrate tissues, probably by removal of calcium from the pectic material of the middle lamella.

The changes in composition which tissues undergo when rotted by organisms, and the differences in composition between resistant and susceptible varieties, have received considerable attention. One of the earliest of such studies was by BEHRENS (**4**), who used a number of fungi, among them *Sclerotinia fructigena*, on apple. REED (**39**) found that *Glomerella* not only decreases the acidity of apples and of synthetic media, but actually makes the latter alkaline. HAWKINS (**28**) found that *Glomerella cingulata* grown on peaches could hydrolyze and assimilate the pentosans, as well as utilize the monosaccharides, but that *S. cinerea* could not utilize the pentosans of the peach (**29**). In the latter case there was an increase in titratable acidity during the rotting. In the case of potato tubers infected with various species of *Fusarium* (**31**) there was a decrease in sucrose, reducing sugars, pentosans, galactans, and dry matter; an increase in crude fiber, due to its formation in the hyphae of the fungi; and no change in the starch and methyl pentosans. BISBY (**6**) and EDSON (**21**) also reported no effect on the starch of potato tubers by certain fungi, and suggest that potato starch could be made from rotted tubers. VALLEAU (**46**) examined many plum varieties as to their content of tannin, but

could find no correlation between this factor and their resistance to brown rot. CULPEPPER, FOSTER, and CALDWELL (**20**) made detailed analyses of apples infested with *Sphaeropsis malorum*, and found that the rotted fruit had undergone considerable loss in dry matter, a loss in alcohol-ether-water-alcohol extractives, an increase in protein nitrogen and in protein phosphorus, a transfer of minerals from the insoluble to the soluble fraction, a loss in total sugars, mostly in the monosaccharides, a decrease in titratable acidity, and a marked increase in alcohol. Starch was not affected.

STEVENS and HAWKINS (**43**) adopted a procedure that elucidates the progressive changes during rotting, by analyzing (1) the fresh strawberry fruits, (2) the sound fruit after storage under the same condition as the inoculated fruit, and (3) the fruit inoculated and rotted by *Rhizopus nigricans*. These three samples show the parallel changes in sound and infected fruit. They found that the acids in the sound fruits decreased, probably due to respiration, and that the acids decreased to a less extent in the rotted fruit. The authors believed this to be due to an interference with the tissue respiration by the fungus and not to the production of ammonia. Sucrose, reducing sugars, and dry matter decreased more rapidly in infected than in sound fruit. The fungus causes the tissue to soften and to become watery, but whether this is due to the death of the cells or to an anesthetic effect is still an open question. STEVENS and MORSE (**44**) reported that in the end rot of cranberries there is a marked decrease in sugars, while the proximate constituents remain fairly constant. The protein, fiber, and ash, however, show such relative increases as would be expected from the loss of dry matter by respiration. GIDDINGS (**22**) and RUSSELL (**40**) reported the inauguration of studies on apple leaves and on potato tubers, respectively, to determine the chemical basis of resistance, but they offer no conclusions as yet.

Recently several papers have appeared which deal with the nitrogen distribution of diseased plant tissues, and which promise to furnish a new line of attack on these problems. BONCQUET (**10, 11**), working with the mosaic disease of tobacco, *Streptococcus solani* on potato, and *B. morulans* on beet leaves, reported that nitrites and ammonia were invariably found in diseased but never

in healthy parts. The amount of nitrites was proportional to the intensity of the pathologic condition. The reducing organisms were mostly in the vascular tissues. The reduction of the nitrates brought about nitrogen starvation, with a consequent yellowing and distortion of the affected tissue. In a field where potatoes had been grown continuously for fifteen years, nearly every vine was affected with nitrogen starvation, although the soil contained an abundance of nitrates. In tobacco mosaic it was observable that the plants tended to oppose these chemical forces both by morphological and by physiological means; thus the secondary organs were reduced in size, more water was transpired, and the oxidizing enzymes showed greater activity.

JODIDI, MOULTON, and MARKLEY (**34**) made a detailed dissection of the nitrogen constituents of spinach mosaic, and found evidences of denitrification, due to the production of nitrites and their subsequent action on amino nitrogen groups. In cabbage mosaic (**33**) a similar condition was found, hence nitrogen starvation is believed to be the cause of the abnormal appearance of the leaves in these diseases. It is to be regretted that BONCQUET and BONCQUET give none of their methods of analysis, nor any data whatsoever in their papers.

Material

Five varieties of plums, grown at the University Fruit Breeding Farm at Excelsior in 1920,[2] were selected for the work. Three of them show marked resistance to the attacks of the brown rot fungus, while the other two are very susceptible. Samples were picked at three stages of growth: (1) when half grown, (2) when fully grown and just beginning to ripen, and (3) when fully ripe, but still on the tree. In most cases each sample was divided into three portions. One portion was analyzed immediately, another was inoculated with a pure strain of *Sclerotinia cinerea* and placed in a moist chamber to rot, and a third portion was placed in a moist chamber without inoculating and left for the same length of time as the corresponding inoculated portion. The inoculations were made by injecting a suspension of spores with a hypodermic syringe into the

[2] Acknowledgments are due to Dr. M. J. DORSEY for assistance in obtaining the material.

tissues of the plums, after the latter had been sterilized with mercuric chloride. The plums were left to rot as long a time as was practicable, which was usually from five to seven days after all the tissue had turned brown to the stone. The same degree of rotting was not obtained in all cases, since this cannot readily be judged.

Methods

PREPARATION FOR ANALYSIS.—In preparing the samples for analysis the stones were removed, the pulps frozen in an ice and salt mixture for three hours, ground in a food grinder, and pressed in a hydraulic press. All manipulations were maintained as uniform as possible throughout the series. The expressed juice was then used for all the subsequent analyses. HARVEY (**26**) has shown that, in order to obtain the true P_H of a juice, the latter should be expressed without freezing, since the freezing precipitates certain proteins and thus changes the H-ion concentration. This fact had to be ignored in the present instance, however, since the determination of the other solutes must be made on juice from frozen tissue, and since the amount of material available was not large enough to admit of two samples of juice being taken in each case. It might be of interest to record the results of a single test of the effect of freezing. The material was some seedling plums about one-third grown.

	Frozen	Unfrozen
Percentage of pulp obtained as juice	70	60
P_H	1.67	1.48

SPECIFIC GRAVITY.—The specific gravity was obtained by means of a Westphal balance, after the juice had stood at least an hour.

TITRATABLE ACIDITY.—Because of the high pigmentation of the juices soon after expression, titration by means of an indicator was impossible; hence the electrical conductivity method was employed. The peak of the curve could be read with an error of about ± 0.3 cc. 0.1 N NaOH.

HYDROGEN-ION CONCENTRATION.—The electrometric method was used for determining the P_H of the juices. Considerable trouble was experienced with the poisoning of the electrode by the

juices, but with care in the renewal of the platinum an accuracy of 0.3 millivolt was obtained.

TANNIN.—The Procter-Löwenthal method as detailed in the Official Methods (1) was used.

OXALIC ACID.—In the case of the juice, 40 cc. were treated with 80 cc. of 95 per cent alcohol, filtered, washed, and made up to 200 cc. Aliquots of this were used for oxalic and for malic and tartaric acids. For oxalic the alcohol was evaporated, the material neutralized with ammonia, acidified with acetic acid, filtered if necessary, treated with calcium acetate in the hot, the calcium oxalate filtered off, and titrated with permanganate. The precipitate no doubt was impure, as it adsorbed some coloring matter, but a satisfactory end point was obtained, and the results are at least comparative. In the case of the residue, 10 gm. was digested with 75 cc. of 0.8 per cent hydrochloric acid for one hour, to liberate any oxalate existing in the residue in the form of the calcium salt. The extract was treated as in the case of the juice.

NITRITES.—The qualitative test with α-naphthylamine and sulfanilic acid was used.

PROTEIN AND NON-PROTEIN NITROGEN.—Trichloracetic acetic acid was used as the protein precipitant. BLISH'S (8) copper hydrate method was tried in comparison with the trichloracetic acid. Since the latter gave the same results and is simpler to use, it was adopted. Twenty cc. of juice plus 20 cc. of 25 per cent trichloracetic acid were allowed to stand overnight, and the nitrogen in a filtrate determined. In the case of the residue a weighed sample was extracted with boiling water, and the filtered extract treated with the protein precipitant. It was realized that in the case of the residue two factors for precipitating proteins were used, heat and the trichloracetic acid. This fact might give incomparable results on the two sets of samples, but there was no other apparent way of getting the data on the residue.

MALIC AND TARTARIC ACIDS.—The optical method (51) for obtaining these two acids in the same solution was used, since it was thought possible that both were present, although BIGELOW and DUNBAR (5) found only malic. If only one of the two acids be present when this optical method is used, the rotation readings

should indicate zero for the one absent. In applying the method to the plum samples, the results in general did give zero values for tartaric. In some cases, however, positive values were obtained, and in others even negative values. Since these anomalies indicated the presence of some interfering substance, it was found necessary to reject all the data on these two acids; hence they are not presented here, although it was deemed well to record this failure of the double polarization method on plum juices.

Experimental data

CHARACTERISTICS OF ROT.—In table I are listed the varieties of plums used, the abbreviations for these varieties used throughout this paper, the dates of taking samples, and the length of time required for rotting in each case. Probably the same degree of rotting was not obtained in all cases, but the relative rates of progress of the fungus attacks are no doubt fairly well indicated

TABLE I

SAMPLING DATA FOR VARIETIES OF PLUMS USED

VARIETIES	ABBREVIATIONS USED	STAGE I, HALF GROWN		STAGE II, FULLY GROWN		STAGE III, RIPE	
		Picked	Days required to rot	Picked	Days required to rot	Picked	Days required to rot
Resistant							
Burbank×Wolf 9	B×W9 or 9	July 12	15	Aug. 25	17	Aug. 31	15
Burbank×Wolf 16	B×W16 or 16	July 12	13	Aug. 20	18	Aug. 28	15
Abundance×Wolf 30	A×W30 or 30	July 12	13	.....	.....	.....	.....
Susceptible							
Compass	C	July 12	9	Aug. 3	11	Aug. 20	12
Sand Cherry×Formosa	SCF	July 12	10	Aug. 3	13	Aug. 11	12

by the figures. It is clear that the varieties listed by the horticulturists as resistant are considerably more slowly rotted than are the susceptible varieties. There is more difference in this respect in the earlier than in the later samples. This is in accordance with the commonly observed characteristics of fruit diseases, that in general they become less resistant as maturity approaches. Because of this, the first set of samples was observed more keenly than the following ones, and some interesting points were noted. In connection with the rate of spread of the rot, the time required for

the surface of the fruits to become brown was recorded, as shown in table II. These figures show the same slower rate of rotting of the resistant varieties.

TABLE II

BEHAVIOR OF VARIOUS PLUM VARIETIES TOWARD ROTTING ORGANISM AT FIRST STAGE OF GROWTH

VARIETIES	DAYS REQUIRED FOR SURFACE TO BECOME BROWN	DAYS REQUIRED FOR COMPLETE ROTTING	RELATIVE ABUNDANCE ON SURFACE OF	
			Hyphae	Spores
Resistant				
Burbank×Wolf 9.....	13	15	++	+
Burbank×Wolf 16.....	7	13	+++	+
Abundance×Wolf 30.....	6	13	+	+++
Susceptible				
Compass.....	5	9	+++	+++
Sand Cherry×Formosa..	5	10	+++	+++

FIG. 1

FIG. 2

FIGS. 1, 2—Fig. 1, dish of Burbank×Wolf 16 plums at end of rotting period in half-grown stage of growth (table II), showing abundance of hyphae on surface and relative scarcity of spore tufts; fig. 2, dish of Abundance×Wolf 30 plums at end of rotting period in half-grown stage of growth (table II), showing abundance of sporulation and scarcity of hyphae on surface.

Another varietal difference was the character of the aerial portions of the fungus, especially as to the relative abundance of spore tufts and of hyphae. These data are recorded in table II. Although the differences among the varieties were well marked, they are not correlated in any striking way with resistance

characters. The susceptible varieties show more abundant aerial growth of both sorts than do the resistant. Possibly the amount of surface growth is dependent on the vigor of the subsurface growth, and the latter is no doubt less in the resistant varieties (figs. 1, 2).

Another varietal characteristic was the relative firmness of the rotted fruit. The sound fruit of the resistant varieties was somewhat more firm than that of the susceptible, particularly in the ripe stage. After rotting, the differences were even more marked. Although this fungus causes what is usually called a firm rot, the rotted fruit of the *C* and *SCF* varieties was almost watery in some instances; while the resistant varieties maintained a firm or even hard texture. This phenomenon may have to do with the character of the pectins, as has been suggested by others. The pectin relations of hosts and parasites offer a fruitful field of investigation (**53**).

Chemical analyses

Instead of presenting the analytical results in tabular form, which would be rather involved and cumbersome, they are given in the form of charts (figs. 3–7). The data could conceivably be grouped in many different ways, so as to show (1) the comparison of the fresh fruit of the several varieties; (2) the progressive changes during the ripening process; (3) the changes involved during storage in the laboratory, both with and without the action of the fungus; and (4) the effect of the rotting process. This would mean four different groupings of the data in four sets of charts. It was decided to limit this to two groupings. The first set, figs. 3 and 4, bring together side by side the data showing the change in composition of the samples during the storage and rotting in the laboratory. The fresh samples in each case are designated *a*, the sound samples stored in the laboratory without inoculation *b*, the rotted samples *c*. In these charts it is easy to follow the changes brought about by the rotting, and the changes taking place during the three stages of growth, by comparing all the *a* samples, the *b* samples, and the *c* samples in each variety.

The second set, figs. 5–7, bring together side by side the data for comparing the various varieties, that is, the *a* samples for all

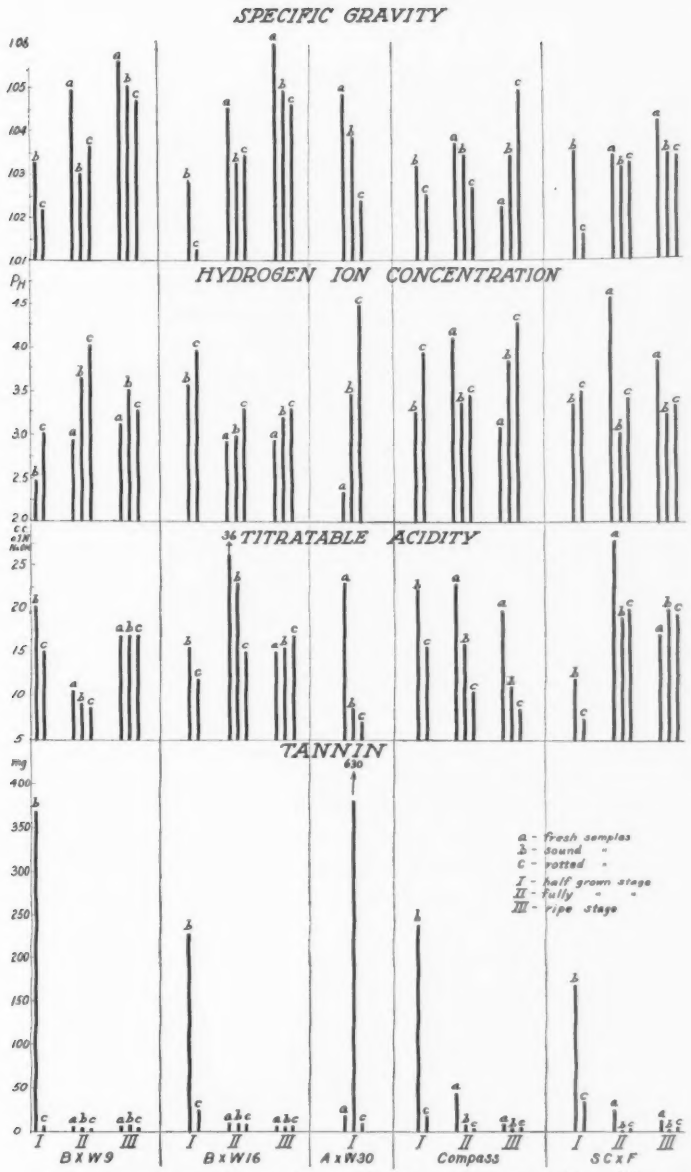

FIG. 3.—Graphs showing specific gravity, P_H values, titratable acidity, and tannin content of juices of all plum samples; figures for titre and tannin are on basis of 10 gm. of juice.

varieties are assembled, then the *b*, and then the *c* samples. In each case the three (in some cases two) resistant varieties are given first, then the non-resistant, in order to facilitate comparison. A brief discussion of each factor will be given.

SPECIFIC GRAVITY.—Referring to the top group of graphs in fig. 3, it will be seen that in most cases the specific gravity of the expressed juice decreases from *a* to *b*, that is, in the sound fruit during storage in the laboratory, and that there is a still further decrease from *b* to *c*, that is, in the rotting fruit. In many cases the decrease in the rotted samples is very marked. Probably respiration consumes sugar in the sound fruit with a consequent decrease in density of juice, and in the rotting fruit the added respiration of the invading fungus causes a still further drop in density. STEVENS and HAWKINS (**43**) noted a similar phenomenon in rotting strawberries. There is one marked exception to this, in the case of the third stage of the Compass variety, but this may be an analytical error.

In fig. 5 there are indications of varietal differences in juice density that may be correlated with resistance properties. Thus varieties 9, 16, and 30 (resistant) in most cases have a higher specific gravity than varieties *C* and *SCF*, and this holds even in the rotted samples. Whether the osmotic pressure of the host sap may be a controlling factor for this fungus is not known. The writers know of no measurements of its tolerance to strong nutrient solutions except the work of HAWKINS (**30**), who tested the ability of a number of fungi to grow on concentrated solutions of sugars and salts. *S. cinerea* would grow on 2.4 M glucose, 1.4 M potassium nitrate, and 0.6 M calcium nitrate. These figures would correspond roughly to 43, 14, and 9 per cent, respectively, which are far higher than any concentrations of fruit juices. It is to be noted, however, that HAWKINS gives no information as to the rate of growth at the various concentrations used; hence it is possible that the differences found in the saps of the plum varieties might account in part for the differences in rate of growth of the fungus.

HYDROGEN-ION CONCENTRATION.—In fig. 3 a comparison of the changes in reaction of the juice can be made, the values being given in terms of P_H. No consistent and striking differences are

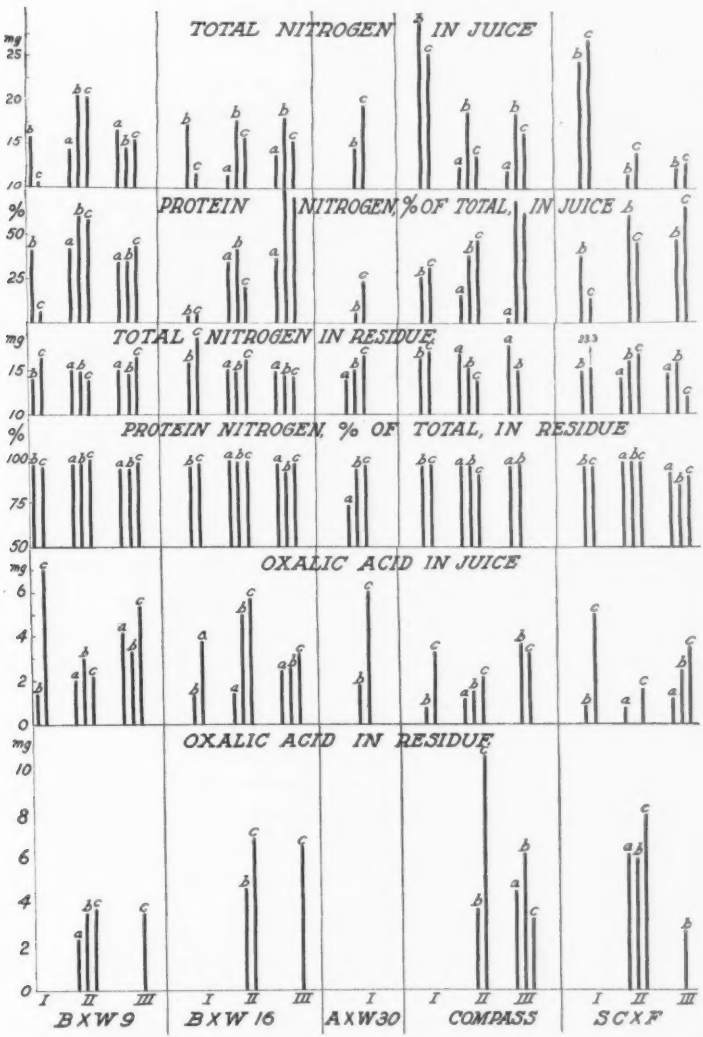

FIG. 4.—Graphs showing total nitrogen, protein nitrogen, and oxalic acid in juices and residues of all plum samples; figures are on basis of 10 gm. of juice and 1 gm. of residue.

discernible, but in most samples the acidity is less in the rotted than in the sound samples. In a majority of cases the fresh material shows a higher acidity than the material after storage in the laboratory, but the data are not conclusive on this point. When the analyses are arranged as in fig. 5, it is seen that in the fresh samples the resistant varieties have a somewhat higher acidity that the non-resistant, but such differences do not obtain in the stored and in the rotted samples. It is also evident that the acidity in plums neither increases nor decreases to any appreciable degree as ripening progresses. This is in accordance with most of the observations on other fruits.

Since the differences in acidity between resistant and non-resistant varieties are not great, and since the growth of the fungus in the tissue tends to lower the acidity to only a slight extent, there is not much evidence that unfavorable acidity is an important factor in resistance of plums to brown rot.

TITRATABLE ACIDITY.—In figs. 3 and 5 the determinations of the titre of the juices follow the trend of the P_H values in reverse order, that is, when the titre is high, the hydrogen-ion concentration is high. There are some exceptions to this, which may be due to differences in buffers in the juices. In fact, in the fresh samples the resistant varieties have both a lower P_H and a lower titre, which would indicate that the acids are much more highly dissociated. The data for oxalic acid show that these varieties do have slightly more of it than the others, but the amounts involved are too small to amount to the differences in H-ion concentration. The character of the buffers may determine this. A careful study of the graphs shows that the changes in titratable acidity are relatively greater than the corresponding changes in H-ion concentration. This becomes more evident when numerical values are used for the comparison. The average percentage decrease in titre from *b* to *c* in all the samples is 17. The average percentage increase in P_H values is 9 (assuming a theoretically possible increase to $P_H = 7$). This would indicate a consumption of acid by the fungus, rather than a production of buffer, in modifying the reaction during rotting. It was hoped that the malic acid determinations would give direct evidence on this point. These determinations will be

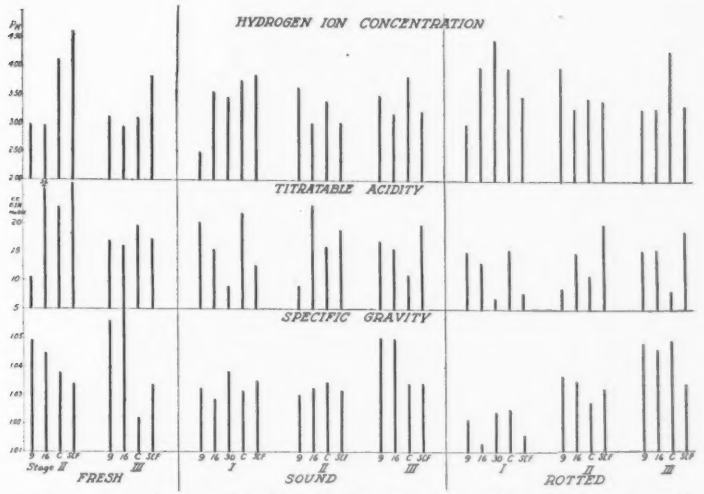

FIG. 5.—Graphs in which data for P_H values, titratable acidity, and specific gravity are assembled to bring together resistant and non-resistant varieties of plums for direct comparison; varieties 9, 16, and 30 are resistant (see table I and fig. 3).

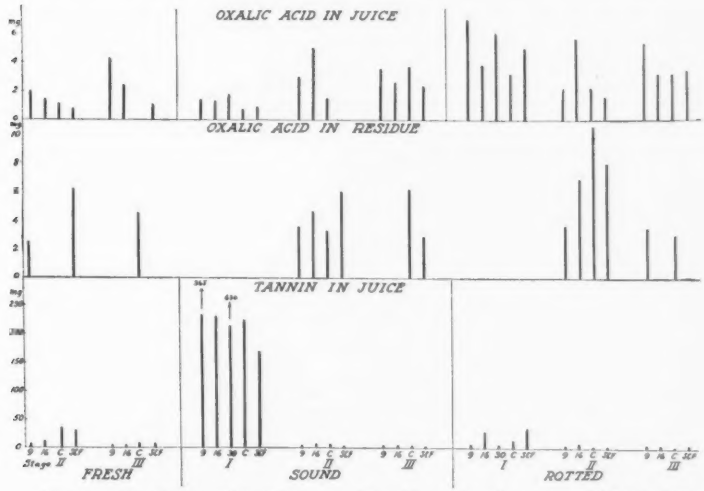

FIG. 6.—Graphs in which data for oxalic acid and tannin are assembled to bring together resistant and non-resistant varieties of plums for direct comparison (see table I and figs. 3, 4).

attempted again by another method. Citrus mottle leaf offers another example of an interesting discrepancy between the titre and the H-ion concentration (**36**).

TANNIN.—The conspicuous fact brought out in figs. 3 and 6 is the great increase in tannin in the *b* samples in the first stage of growth. This indicates that in these half-grown fruits the tannin increases rapidly after the fruit is picked from the tree, but that if the fruit is infected by the fungus the tannin does not increase. COOK and his colleagues (**3, 16, 17**) report an enzyme that forms tannin rapidly either when the fruit is picked or when it is wounded. The former fact is corroborated in the present work, but not the latter. In fact, infection by the fungus not only does not cause an increase in the tannin content, but in varieties *C* and *SCF* in fig. 3 there is a decrease over the fresh samples. Two facts should be kept in mind in this regard: first, that the great increase in tannin after picking from the tree occurs only in the half-grown stage of growth; and second, that the decrease in tannin in the rotted samples is noticeable only in the two later stages of growth, since unfortunately in the first stage the fresh sample was analyzed only in the case of $A \times W30$. In fig. 6 it can be seen that in the fresh samples of the second stage the resistant varieties have a lower content of tannin than the susceptible, and that in the sound samples of the first stage the facts are reversed. It is difficult to perceive any facts that can be correlated with resistance characters. VALLEAU (**46**) came to the same conclusion.

OXALIC ACID.—Figs. 4 and 6 give the analyses for oxalic acid in the juice and in the residues from the juice. In all cases there was a small amount of oxalic acid present in the juice of the fresh fruit, as judged by the reduction of permanganate. It does not average over 0.02 per cent of the juice. In most cases there is more oxalic acid in the *c* than in the *a* or *b* samples, indicating that during the rotting a production of the acid takes place. This is in accordance with the findings of COOLEY (**19**), who reported that oxalic acid was produced by *Sclerotinia*. The amount of oxalic acid produced in the rotted plums, however, seems insufficient to exert any very marked solvent power on the tissues. Although the data for oxalic acid in the residues are very incomplete, they indicate

the same general trend as do those for the corresponding juices. There are some indications in fig. 6 that the resistant varieties have a higher oxalic acid content than the susceptible, both with and without fungus action in the tissues. If this is found to be the case in future analyses, it may constitute some new evidence on the question of resistance properties.

NITROGEN DISTRIBUTION.—In figs. 4 and 7 are presented the results of the analyses for total and for protein and non-protein nitrogen. There is great irregularity in the quantities of total nitrogen in the juice in the three groups of samples, so much so

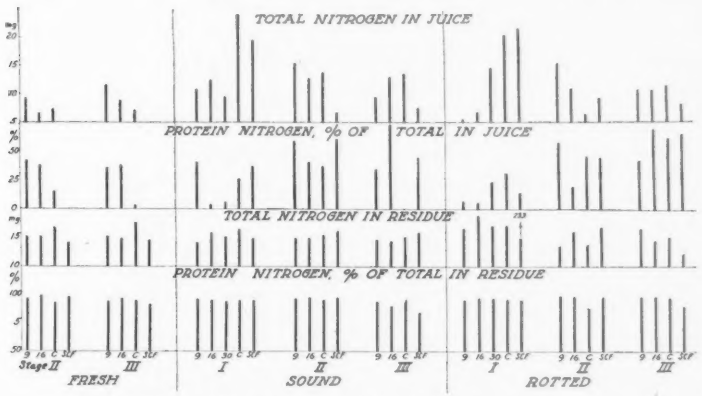

FIG. 7.—Graphs in which data for total and protein nitrogen are assembled to bring together resistant and non-resistant varieties of plums for direct comparison (see table I and fig. 4).

that it is difficult to see any definite trend to the graphs in fig. 4. In the case of the residue, there is some evidence that the rotted samples (*c*) have a greater amount of total nitrogen than the sound (*b*). This is no doubt due to the facts that a far greater proportion of the nitrogen is in protein form in the residue, and that the building of fungus protein makes this protein nitrogen still higher in the residues of the rotted samples. No definite trend nor significance can be seen in the data for the protein nitrogen in the juice.

NITRITES.—No test for nitrites was obtainable in any of the samples. The disturbance of the nitrogen nutrition of the host

cannot be a factor in this disease as it seems to be in others (**10, 11, 33, 34**). No varietal differences in the nitrogen content and forms of nitrogen are discernible in fig. 7.

Discussion and summary

The laboratory inoculations recorded in this paper corroborate the field observations on plum varieties as to their relative resistance to the brown rot fungus, *Sclerotinia cinerea*. In the field an important factor in resistance is the thickness of the skin. In the present studies this was eliminated by injecting the spores into the tissues, so that the differences in the rate of rotting were due mostly to physiological factors. The object of the investigation was to throw some light on these factors. The varieties showed not only different rates of rotting, but the character of the growth of the fungus differed as to the amount of fruiting. The susceptible varieties in general showed a greater amount of fruiting on the surface of the fruits. No study is recorded in the literature of the factors affecting sporulation in this fungus, except the vitamine relations touched upon by one of the writers (**52**). In the present work it was noticed that the juices of resistant varieties have a higher specific gravity, a slightly greater H-ion concentration, a lower titratable acidity, and a slightly greater oxalic acid content. In these items the differences between resistant and non-resistant varieties are not sufficiently marked to convince one that they constitute the chemical basis of resistance. Culture work with *Sclerotinia*, using fruit juices in which the various factors can be varied and controlled, will no doubt throw considerable light on the question.

When the fungus rots the plum, some well marked changes in composition take place in the tissues. The juices show considerable decrease in specific gravity, a decrease in true acidity, a decrease in titratable acidity that is of greater magnitude than the decrease in true acidity, and an increase in oxalic acid content. The fungus in some way prevents the production of tannin that takes place in the green fruit after it is picked from the tree. The fungus converts a portion of the non-protein nitrogen of the host into protein nitrogen in its own mycelium.

Nitrites could not be detected in any of the samples. They are probably not a product of the rotting by this fungus. No hypothesis can be suggested as yet for the chemical and physiological basis of resistance in the brown rot of stone fruits.

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VARIATIONS IN CYTOLOGY AND GROSS MORPHOLOGY OF TARAXACUM

I. CYTOLOGY OF TARAXACUM LAEVIGATUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 293

PAUL BIGELOW SEARS

(WITH PLATES IX, X)

Introduction

These investigations are the outgrowth of a study, begun in 1914 at the instance of the late Professor C. E. BESSEY, of parthenogenesis in *Taraxacum vulgare* (Lam.) Schrk. and *T. laevigatum* (Willd.) DC. These two species are the common ones in central United States, being respectively designated as *Leontodon Taraxacum* L. and *L. erythrospermum* (Andrz.) by BRITTON and BROWN (3). In 1917 the existence of ameiotic parthenogenesis in both species was confirmed (23) and certain pollen abnormalities briefly described. These abnormalities have invited more critical analysis as a means of throwing light upon certain phases of variation and degeneracy, and likewise upon the problem of synapsis. The study of non-cytological variations has been directed largely to leaf characters. This is due to frequent references in the literature of *Taraxacum* to "polymorphy" and to the wholesale erection of species (cf. Index Kewensis 10).

Maturation phenomena in embryo sac and pollen have been homologized and found to be highly fluctuating. The fluctuations, instead of being anomalous, seem plainly to indicate variations in the duration and relative intensity of (1) chromosome individuality, (2) sex, (3) polarity.

Synapsis in the sense of chromosome pairing is expressed with varying degrees of vigor and quite without reference to doubleness of the spireme thread.

Leaf variation within the species is shown by quantitative studies to be a matter of senescence and rejuvenescence. The rôle of environment is secondary. The interplay of senescence and

environment is sufficiently potent to have produced from a few valid species transient forms fitting many published descriptions given specific or varietal rank. Attempts to correlate degree of leaf dissection with internal anatomy have not succeeded, but senescence and dissection are accompanied by an increase in carbohydrates as compared with nitrogenous substance.

Numerous colleagues, and in particular Professor JOHN H. SCHAFFNER of Ohio State University, have given generous help whenever called upon. It seems proper also to express appreciation of the difficulties confronting earlier workers whose conclusions, and in some cases whose observations, have not been confirmed here.

IDENTITY OF SPECIES.—This plant, known as the "red-seeded dandelion," is without doubt cosmopolitan. It is listed as *T. erythrospermum* Andrz. by GRAY (**19**), and as *Leontodon erythrospermum* (Andrz.) Britton by BRITTON and BROWN (**3**). SEARS (**23**), as well as SHERFF (**25**), for reasons that will appear later, has accepted the decision of HANDEL-MAZETTI (**6**) with respect to nomenclature.

BRITTON employs the generic name of *Leontodon* upon the authority of LINNAEUS' Sp. Pl. 798 (**14**). HANDEL-MAZETTI in his monograph presents the tabulation and critique of authorities upon which he bases his selection of the name *Taraxacum*. It is convincing. Incidentally he makes clear why *Leontodon Taraxacum* L. cannot stand as a valid species name because of incomplete diagnosis.

The species with which we are concerned here is discussed by the same authority as follows:

> Was den Namen anbelangt der bereits auf die diversisten Pflanzen angewendet wurde, so konnte ich mich an dem schön fruchtenden Originalexemplar Willdenows von seiner Bedeutung überzeugen. . . . . *Leontodon laevigatus* Willdenow, 1800; *Taraxacum laevigatum* De Candolle, 1813; *T. erythrospermum* Besser, 1822.

The last citation (quoted from the tabulation of synonyms on p. 109) is doubtless the one upon which GRAY and BRITTON base their specific nomenclature. BRITTON'S citation for the species is ANDRZEJOWSKY in Bess. Enum. Pl. Vilh. 75. 1821. For further critical discussion the careful paper by SHERFF (**25**) may be cited.

REPRODUCTION.—The more important studies of reproductive physiology and morphology in *Taraxacum* are so well known and so frequently cited that they require only brief comment here.

1896. SCHWERE (**22**): embryo arises from egg in sac of typical appearance.

1900. ANDERSSON and HESSELMAN (**1**): pollenless arctic specimens produce fruit, parthenogenesis suspected.

1903. RAUNKIAER (**18**): "Species Danicae Taraxaci castratione agamice propagari demonstratum est; species omnes Taraxaci semper parthenogenetice propagari verisimile est."

1904. KIRCHNER (**13**): pollen of *Taraxacum* never found germinating upon *Taraxacum* stigmas, although often abundant.

1904. MURBECK (**15**): confirms findings of SCHWERE, RAUNKIAER, and KIRCHNER. Finds embryos in unopened, pollenless flowers.

1904. JUEL (**11**): embryo sac maturation reduced to single division. Apparently no reduction, although prophase resembles heterotypic.

1905. JUEL (**12**): compares maturation phenomena of sexual *Chicoraceae* with those of *T. officinale* (*vulgare?*). Notes double thread in prophase of former but not in latter; former shows haploid number of bivalents in diakinesis, while *Taraxacum* shows diploid number (24–26) of univalents. These facts believed to favor the parasynaptic view of reduction. Following diakinesis in *Taraxacum*, nucleus is believed to elongate and chromosomes to split temporarily, the sequence being regarded as a shift from heterotypic to homotypic prophase. Pollen goes through reduction forming 12 or 13 bivalents in first prophase, but doubleness of spireme not observed.

1907. HANDEL-MAZETTI (**6**): noteworthy monograph of genus. Emphasizes genetic importance of parthenogenesis, but believes RAUNKIAER'S conclusion too sweeping. Shows clearly that pollen development must be highly variable.

1907. DAHLSTEDT (**4**): notes presence of numerous sterile seeds and surmises that normal eggs may be found in otherwise parthenogenetic heads. Also believes sexual species likely to exist.

1909. ROSENBERG (**20**): compares chromosome conditions of *T. officinale* (*vulgare?*) and *T. confertum*, finding in the latter a typical reduction from 16 to 8.

1910. IKENO (**9**): reports *T. platycarpum* Dahlst. to be sexual, while *T. albidum* Dahlst. (white-flowered) is not.

1912. SCHORBATOW (**21**): confirms previous findings for *T. officinale* Wigg. Takes liberal cognizance of cytological variation.

1913. OSAWA (**17**): compares in detail cytology of species studied by IKENO, agreeing in general with JUEL'S conclusions. Finds a variable degree of pairing in pollen diakinesis of *T. albidum*, and besides normal maturation of tetrads, the formation of diads by "homotypic" division. Notes amitosis and supernumerary nuclei in pollen; also 16 and 8 chromosomes in sexual, 36 to 40 in parthenogenetic species. Parthenogenesis probably due to hybridization.

1917. SEARS (**23**): *T. laevigatum* as well as *T. vulgare* shows ameiotic parthenogenesis. The former generally gives higher percentage of sterile fruits, and both exhibit pollen abnormalities, including extrusion of chromatin, amitosis, and defective spindles.

1920. STORK (**26**): *T. laevigatum* is ooapogamous, and embryo sac maturation agrees in general with accounts of JUEL and OSAWA for other ooapogamous forms. Also 26 to 30 chromosomes found, but said not to split during the elongated phase which is believed to follow diakinesis.

RELEVANT CYTOLOGICAL PROBLEMS.—As suggested earlier, those of chief interest in connection with the present study are (1) the mode of synaptic pairing, and (2) cytological variation.

SYNAPSIS.—The conclusion of workers already quoted (**12**, **17**), who have compared ameiotic species of *Taraxacum* with related sexual species, favors the parasynaptic interpretation of reduction division. Such conclusion is doubtless justified if the observations of prophase conditions upon which it is based are unexceptionable.

The development of thought upon the subject of synaptic pairing has been fully treated by numerous workers, the present trends of botanical opinion being fairly crystallized in papers by DIGBY (**5**) and SHARP (**24**). It is unfortunately true that various questions involved hinge upon observations made near the limit

of resolving power of the microscope. This introduces what should frankly be recognized as a potential source of error, and dealt with accordingly. In undertaking this study it was hoped that the more obvious sequence of events in a parthenogenetic plant might afford a check upon observations necessarily made under conditions of optical difficulty. This hope has at least been partially justified.

VARIATION IN CELL PROCESSES.—Cytologists have, through no fault of judgment, been so charged with the duty of learning the normal sequence of events in plants that as a rule they have given little attention to "anomalies." Even the most conservative theories of the cell as a physico-chemical mechanism must admit the likelihood of considerable fluctuation in its processes. WASIELEWSKI (**27**) emphasizes the phylogenetic continuity between mitosis and the types of amitosis produced by chloroforming meristem. Moreover, the results obtained by NATHANSOHN (**16**) in producing abnormal division types by the use of ether are highly suggestive, when viewed either in the light of modern theories of anaesthesia or of such work as that of BONNS (**2**). The latter has clearly shown a marked increase in proteolytic enzyme activity as a result of etherization. The experiments of HOTTES (**8**), demonstrating powerful effects of temperature change upon the spindle mechanism, are likewise significant. They become peculiarly so in connection with the intimate relation of temperature to enzyme activity.

Careful study and classification of variations in cell behavior have already yielded data of interest in genetics, and they may afford the clue to an isolation and analysis of the factors involved in cell behavior, which are now known, so far as they are recognized, by terms so general as to be noncommittal.

Procedure

All material studied was collected from plants which had been identified after fruiting. A wide range of fixing reagents was tested, including mixtures of absolute alcohol and glacial acetic acid in various proportions. The best results were obtained with a solution of two parts of alcohol and one of acid. Beautiful

preparations were obtained by the use of Flemming's stronger fixing fluid. In comparison with acetic alcohol material these showed little difference in nuclear condition, but inspection of the cytoplasm made it very evident that the electrolytes contained in Flemming's solution had caused violent coagulation of certain cell colloids. This circumstance should militate against its use in critical work. Acetic alcohol kills almost instantly, and contains two components whose effects are mutually corrective. From the theoretical standpoint of modern colloid chemistry it ought to be a very desirable reagent. Numerous experiments, as well as the variety of formulae which have been recommended by different workers, suggest strongly that failures with it have often been due to use of unsuitable proportions of the components.

Sections were cut from 6 to 12 μ in thickness, and were stained with iron-alum, alone and with counterstain, and with Flemming's triple solution. Drawings, unless otherwise noted, were made with Spencer camera lucida through Bausch and Lomb binocular equipped with no. 12 compensating oculars and 1.9 fluorite objective.

It should be noted that the use of the word synapsis in this paper has been limited to the matter of synaptic pairing. The term synizesis is used throughout to designate the balling of chromatin in early prophase.

Observations

SOMATIC DIVISIONS.—These were observed very frequently in all stages, in nucellar and other meristem. So far as can be determined, they present no unusual features. The chromosomes segment as curved rods from a fairly thick and quite uniform spireme, and are certainly about twenty-six in number.

MATURATION DIVISIONS.—The earliest stages that could be identified were marked by an enlarged nucleolus and not more than thirteen paired centers of chromatin aggregation. These are shown in figs. 1 and 2, and can fairly be construed as prochromosomes. JUEL (12) does not mention them, while OSAWA (17) describes them for both the species he studied as originating by an increase in size and number of the granules upon a linin meshwork,

that is, from a resting nucleus as conventionally described. Observations here lead to agreement with SHARP (**24**), as follows:

> While one easily gains the impression of separate chromatin granules connected by threads with another substance . . . . it seems more probable that the "chromatin granules" are merely the heavier portions of the alveolated and reticulated chromosomes, and that the lighter "supporting network" consists simply of the thinner portions of the same, together with the delicate anastamoses.

In portions of the thread as it enters synizesis, a curious partly paired, partly vacuolate-split appearance is discernible (fig. 3). It is stages of this sort which lend themselves as conveniently to one philosophy of nuclear division as another, and which should not be taken as pivotal until all other means of explanation have been exhausted. The optical difficulties attendant upon the close state of aggregation are very great here of course, necessitating thin sections and special technique.

Synizesis culminates in an extremely dense ball whose components are without doubt filiform. The embryo sac mother nucleus in fig. 4 is typical in every respect save that of size, being larger than usual. The loosening thread (dolichoneme) is fairly uniform at first (figs. 5, 6), and JUEL (**12**) is doubtless right in stating that any apparent nodes are optical effects due to foreshortening or crossing.

After the thread becomes rather evenly distributed through the nuclear cavity, its uniform appearance is altered by the advent of changes which are hard to explain except as fissions. Certainly they are quite different from (1) accidental or other juxtaposition of whole threads, or (2) the twisting together of limb and bight into a loop. Both (1) and (2) are to be seen in figs. 7 and 8, where they may be compared with the seeming fissions. Whatever the change that gives this appearance of duality, it is clearly not simultaneous throughout the thread. A priori, is there any reason why it should be? The unevenness of its origin perhaps may explain the failure of other students of parthenogenetic species of *Taraxacum* to observe anything suggesting a "double" thread. Careful inspection of OSAWA'S fig. 46 (**17**) shows that the phenomenon is by no means precluded there.

Instead of occurring uniformly, thickening of the thread seems to be accompanied by the beginnings of its segmentation. Figs. 9 and 10 show nodes whose structure is seemingly homogeneous. On the other hand, it is always possible to find some showing clearly a longitudinal duality, even culminating in a divergence or forking of the two attenuate ends. If this appearance of doubleness were visible in every node and the divergent internodes were not visible, one would be justified in seriously questioning the validity of the interpretation. It might then be simply the lateral shadowing normal to translucent cylindrical bodies. If, however, there is really duality, the separating plane in certain nodes must lie more or less parallel to the section and hence not be visible. This would account for those nodes whose appearance is homogeneous.

The nodes rapidly shorten a ıd become truly homogeneous, only the bifurcate internodes remaining as evidence of the double origin of each chromosome. As in all chromosomes, there are occasional lateral projections in addition to the forking internodes, due doubtless to imperfect retraction of pseudopodia at some time during aggregation. The papers already cited (**12, 17, 26**) evince little proof of close attention to this stage, a circumstance doubtless due to its transient character. Figs. 11–13*b* show it in varying aspects. Fig. 12 suggests a rough correspondence between this phase and the so-called second contraction. Certainly the thread shortens greatly, and the chromosomes as they first cut apart are no longer peripheral, but in the nuclear interior.

The bifurcations at each end of the chromosome are not retracted at once, but may shift slightly in position. This gives the appearance of pseudopodia, generally four in number. STORK, OSAWA, and JUEL have all more or less plainly figured but not accounted for these pseudopodia. Fig. 14*b*, as those of the authors cited, shows that after the chromosomes drift to the nuclear membrane and become peripherally oriented, the quadruple projections tend to move to the side of the chromosome away from the membrane. These, with other irregular projections of earlier or later origin, may constitute the "fringe" referred to by JUEL and figured by OSAWA.

Counts at this stage, and also at the somewhat later one resembling diakinesis, show twenty-six chromosomes. This agrees

with JUEL's (12) counts for *T. officinale*. The count registered by STORK for the species now under discussion is twenty-six to thirty. Since the latter takes no cognizance of fission at any time before metaphase, it is possible that his higher estimate is due to reckoning separated halves as units. It will be recalled that the somatic number is about twenty-six, and that there are about thirteen pairs of prochromosomes. Fig. 22 shows a normal reduction division of thirteen univalents at the homotypic plate. These facts all give the necessary assurance that in prophase we have the origin of the diploid number of univalents, unpaired, from a dual and therefore a split thread.

To summarize developments thus far, there is first the appearance of approximately thirteen (the haploid number) pairs of prochromosomes. The thread entering synizesis shows in places a doubleness unexplainable at present. The thread emerging from synizesis becomes very evenly distributed through the nucleus, and then shows what is interpreted as non-simultaneous splitting. By the time segmentation is reached splitting becomes indubitable, and the formation of twenty-six univalent chromosomes occurs by the lateral refusion of the two halves previously split apart.

In contrast with these findings it should be noted that JUEL, OSAWA, and STORK, working on parthenogenetic species of *Taraxacum*, all expressly state that the postsynizetic thread is single and remains so, and that the univalent chromosomes are single in composition. JUEL and OSAWA, working on sexual plants of the same or nearly related genera, report an obvious doubleness of the thread. Since there is no question of the duplex nature of bivalent chromosomes in sexual plants, these investigators conclude that the doubleness noted is due to synaptic pairing. The three workers cited agree that diakinesis is followed by the greatly elongated nucleus as mentioned. STORK, however, considers the chromosomes here to be unpaired, that is, unsplit because "there are certainly not upward of sixty." Accepting his maximum count of thirty as correct, one would scarcely expect to find more than sixty halves.

Comparison of figs. 12–15 with figs. 36–41 strongly suggests that the elongated nucleus is not the outcome of diakinesis, but a

divergent form of it. The chromosome forms in the two sets of figures show no interrelation, but a common origin at segmentation.

It will particularly be noted that connections between chromosomes persist in the beginnings of the elongated stage but not in diakinesis, making it quite unlikely that the former is a derivative of the latter, but not militating against the idea that both are derivable from late segmentation.

The nucleus in the elongated stage is often lobed, moreover, as in fig. 40, and constricted and binucleolate as in fig. 41. In short, the elongated nucleus with about twenty-six *X* and *V*-shaped chromosomes must be regarded as part of a distinct sequence

TABLE I

Stage	Sequence *A*	Sequence *B*	Sequence *C*	Sequence *D*
Prochromosome	13 pairs	13 pairs	13 pairs	13 pairs
Synizesis	Normal	Normal	Normal	Normal
Loose skein	Normal	Normal	Normal	Normal
Splitting	Visible	Visible	Visible	Visible
Segmentation	26 cuboids	26 cuboids	26 cuboids	26 *X*'s and *V*'s; nucleus long
Synapsis	Prompt	Slow	Slow or none; nucleus long	None
Orientation	Compact	Loose	Irregular	None; nucleus lobing
Spindle	Fibers to bivalents	Fibers to univalents	Defective	None
Metaphase	Qualitative, narrow	Quantitative, broad	None or very irregular	Amitosis
Second division	Quantitative, homotypic	Quantitative, somatic (or none)	None, irregular or amitotic	None or amitotic

arising from segmentation and culminating in amitosis, and not as a curious step in the normal maturation process. This amitotic type of development may be designated as sequence *D* (table I). It is illustrated in figs. 36–42.

Returning to diakinesis with its twenty-six cuboid chromosomes, this stage may develop further in any one of the three ways outlined in table I, and designated as sequences *A*, *B*, and *C* respectively. Type *A* is illustrated in figs. 16–22. Here pairing is end to end, following diakinesis, and is both prompt and complete. Orientation is uniform and compact, resulting in a heterotypic metaphase

with thirteen bivalents. This sequence has been completely followed in pollen through heterotypic mitosis and the ensuing homotypic division where (fig. 22) thirteen univalents show. In the embryo sac it has only been traced with definiteness through the compact orientation stage (fig. 17), while fig. *21a*, *b* represents a badly masked anaphase showing thirteen chromosomes at each pole. In addition, it is likely that the metaphase shown by STORK in his fig. 16 is heterotypic, since it agrees with the general aspect of such a stage as found frequently in pollen. Whether the second (quantitative or homotypic division) can occur in the embryo sac as it does in the pollen maturation, giving true reduction, is not known. Inspection of hundreds of embryo sacs failed to disclose tetrad formation, and yet the large numbers of empty fruits in *T. laevigatum* may eventually be explained by occurrence of reduced embryo sacs, never fertilized, quite as much as by the occurrence of amitosis of sequence *D*.

Sequence *B* is shown in figs. *23–30b*. It is ostensibly the sequence which results in reproduction, inasmuch as it is the only mechanism found which in the absence of fertilization insures preservation of the constant chromosome equipment characterizing the species. In this sequence the nuclear membrane disappears before synapsis and orientation are complete. Synaptic pairing is end to end, but takes place so slowly that spindle fibers become attached to each of the halves of each univalent instead of to the univalent as a whole. In consequence the pairs come to metaphase thirteen in number, but with components still end to end and transversely oriented. The resulting spindle (figs. 27, 28) is much broader than that of sequence *A*, and the division is quantitative instead of qualitative, if ordinary canons be right. The partial or delayed pairing here was noted by OSAWA in the pollen of *T. albidum* only, did not attract the attention of STORK, and seems to have been interpreted by JUEL (**12**) as a splitting. HOGBEN (**7**) has described similar phenomena (delayed synapses) in parthenogenetic animals, while the present observations are amply verified by numerous counts made throughout the sequence. Sequence *B* in pollen seems to result in diads of fairly uniform nature, which as a rule do not undergo further growth. In the embryo sac it likewise

produces a diad. One of the cells, usually the apical, disintegrates, while the other develops into an eight-nucleate sac by regular vegetative mitoses. A prophase of the first of these mitoses is shown in fig. 30*a*, *b*, with twenty-six somatic chromosomes segmented.

Sequence *C* is illustrated in figs. 31–35. It comprises a rather wide range of gradations in behavior, completely bridging the gap between types *B* and *D*. Following segmentation, the nucleus elongates, and the membrane disappears, with the twenty-six cuboids widely scattered and quite unpaired. As the spindle fibers appear, orientation and pairing are quite variable in their degree of perfection. In fig. 32 spindle, synapsis, and orientation seem rather perfect, excepting that chromosomes from the extreme ends of the nucleus have been caught at the poles and will doubtless remain there. In other cases pairing cannot be detected, and the majority of the cuboids may be caught at the poles, only a few or none reaching metaphase position. These latter constitute the "delayed" chromosomes familiar in descriptions of pollen abnormalities, although actually the lagging ones are those at the ends. Obviously it is but a short step from this condition, where no cuboids reach metaphase, to amitosis as already described for sequence *D*.

Sequence *C* is best exemplified in the pollen. In the embryo sac it has been traced through orientation. With amitosis it shares most of the responsibility for pollen abnormalities recorded in a previous paper. The chromosomes which never reach metaphase position are likely to be reorganized into nuclei before those at the center reach the poles. These latter "delayed" chromosomes then reorganize as supernumerary nuclei. Additional causes of supernumerary nuclei are (1) irregular lobing during first amitosis, (2) a second amitotic division, (3) extrusion of chromosome substance and formation of membranes about it.

It should be understood that the four sequences described intergrade almost insensibly. It should also be noted that sequence *D* in its extremest fluctuations shows nuclear elongation and amitosis beginning so soon after synizesis that the chromatin has no opportunity to organize beyond the condition of a granular thread.

Discussion

The more important implications of these findings fall under (1) relation to previous hypotheses explaining maturation in parthenogenetic species of *Taraxacum;* (2) effect upon interpretations of normal reduction division (particularly as to synapsis) which have been based upon comparisons of sexual and parthenogenetic species of *Taraxacum;* (3) elucidation of the findings themselves in terms of the fundamental cell activities involved.

1. JUEL'S (**12**) hypothesis, accepted in more or less modified form by subsequent workers, is that maturation in *Taraxacum officinale* begins as a heterotypic and shifts to a homotypic division. As previously stated, this is based upon his belief that the elongated nucleus with *X*-shaped chromosomes follows diakinesis and precedes spindle formation. Since, as has been indicated, the elongated nucleus is a member of a distinct sequence, the hypothesis is placed upon the defensive. Barring this discrepancy, however, the type of division described by JUEL is essentially that of sequence *B*, the type effective in reproduction. It might appear that this is virtually homotypic, since quantitative, and therefore mainly if not in detail in agreement with JUEL'S theory. Possibly this is true, but number and character of chromosomes do not correspond with those usual in homotypic divisions. Sex as a factor is completely absent in homotypic division, while here it is present, in abeyance of course, but potential. This is evidenced by (*a*) chromosome number, (*b*) pairing of prochromosomes, (*c*) synaptic pairing (albeit delayed) of the cuboids, (*d*) occasional cases of true reduction in pollen and presumably in embryo sac. It seems, therefore, that the designation "ameiosis," or "amiosis," proposed by SEARS in 1917 (**23**), and indicating a type of maturation which obviates necessity for subsequent fertilization, is to be preferred to "homotypic mitosis," a term of very explicit implications.

2. The parasynaptic interpretation of reduction division, so far as normal sexual species of *Taraxacum* are concerned, was favored by the work of JUEL and OSAWA, since both workers noted a duality of spireme thread in meiotic and none in ameiotic plants. Closer scrutiny previous to segmentation has revealed a duality in the segmenting thread, while precise counts have indicated that this

duality represents a splitting and not a pairing. These facts seem sufficient to warrant more critical comparison of prophases in sexual and parthenogenetic species of *Taraxacum* before deciding that parasynapsis is actually the source of duality in spireme threads of the former. Moreover, the completed synapsis in sequence *A*, as well as the delayed pairing in sequence *B*, is end to end, rendering any assumptions still more difficult.

3. We have seen that ameiosis does not involve the complete elimination of sex. Rather it involves a retardation and partial inhibition of sex expression. The least degree of inhibition gives us sequence *A*, practically a normal reduction division with synaptic mates pairing only a little more slowly than is usually the case. A greater degree of inhibition obviously occurs in sequence *B*, the delay being more marked. Whatever the ultimate cause of such delay, there can be no question that it amounts to a persistence of chromosome individuality, which at segmentation supersedes the individuality of the nucleus as a dominant phase. The nature of sex inhibition in sequence *C* is more complex. Synapsis is slow and of varying perfection. It is marked by an elongation of the nucleus, clearly indicating a premature expression of polarity. We may conclude, therefore, that encroachment upon sex is progressively increasing.

Type *D* is readily interpreted, in view of these intermediate conditions, as the still earlier and more powerful expression of polarity at the segmentation stage. Not only does the nucleus become greatly elongated and eventually pulled apart, but the spireme split begun in prophase is never even temporarily overcome by the forces making for chromosome individuality. This is evidenced by the presence of *X* and *Y* forms, already noted. Such interpretation of the amitosis in sequence *D* by no means vitiates any possibility that it may be a matter of emulsification, as suggested by coupling the work of NATHANSOHN and that of BONNS. It merely involves a third, and not unreasonable factor, enzyme action, as a means of upsetting the delicate balance between the forces which we ignorantly know as individuality, polarity, and sex. That the dominance of polarity is not likely to be perfect seems probable from the nature of the factors which it overrides.

We have therefore a theoretical right to expect such phenomena as chromatin extrusion, irregular lobing, etc. The supernumerary nuclei produced by such means are thus quite a secondary phase of pollen degeneracy.

Summary

1. Maturation in *Taraxacum laevigatum* differs from that previously described for parthenogenetic species of *Taraxacum* in early prophase, chiefly by showing a split thread from which twenty-six univalent chromosomes segment.

2. Following segmentation, there may be any of four intergrading sequences instead of a single uniform sequence as described for other parthenogenetic species of *Taraxacum*.

3. These sequences are: *A*, almost typical reduction division characterized by perfect end to end pairing of the univalents; *B*, a qualitative division resulting in diads from which the functional embryo sacs arise and for which the term "ameiosis" is proposed, and in which sequence the univalents are slow in pairing; *C*, a more or less irregular division in which pairing of univalents is variable, accompanied by premature elongation of the nucleus and defective orientation; *D*, amitosis in which the nucleus elongates very prematurely and the split thread persists after segmentation, giving twenty-six *X* and *Y*-shaped chromosomes. There is no spindle.

4. These variations are not anomalous, but are traced to an increasing degree of inhibition of sex by other forces, to wit, chromosome individuality and polarity.

5. JUEL'S (12) interpretation of maturation in *T. officinale*, that it begins as heterotypic and switches to homotypic, does not apply in the present case.

6. Evidence for parasynapsis in Chicoraceae, so far as predicated upon the presence of a dual thread only in sexual species, must be reexamined.

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EXPLANATION OF PLATES IX AND X

All figures show a magnification of about 1600, excepting fig. 25, which is about 1200.

FIG. 1.—Complete view of very early E.S.M.C., showing about thirteen paired centers of chromatin aggregation.

FIG. 2.—Partial view of similar nucleus.

FIG. 3.—E.S.M. nucleus entering synizesis, showing curious partly paired, partly vacuolate appearance of chromatin masses.

FIG. 4.—Unusually large E.S.M.C. at climax of synizesis.

FIG. 5.—E.S.M.C. emerging from synizesis, showing uniform character of thread.

FIG. 6.—The same, somewhat later.

FIG. 7.—The same, thread becoming less homogeneous.

FIG. 8.—The same, thread showing dual character in places.

FIG. 9.—The same, segmentation beginning, thread thicker and dual character obvious in most nodes.

FIG. 10.—P.M.C. showing segmentation and dual nodes.

FIG. 11.—E.S.M.C., chromosomes becoming homogeneous, duality mainly visible at internodes.

FIG. 12.—The same, showing origin of cuboid chromosome form; thickening of thread producing contraction of mass toward center.

FIGS. 13*a*, *b*.—Complementary sections of same E.S.M. nucleus, with twenty-six (diploid number) chromosomes, cuboid and with traces of dual internodes still showing.

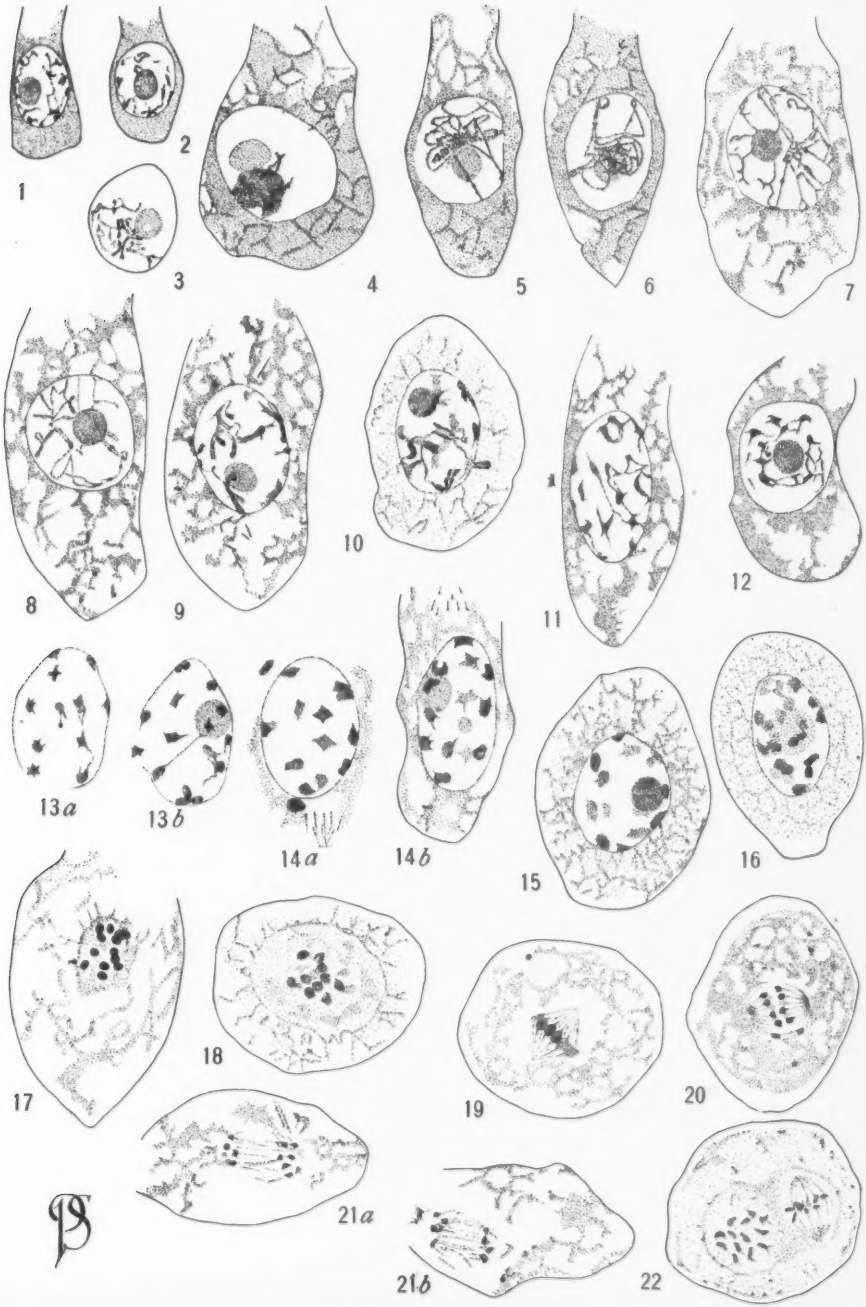
FIGS. 14*a*, *b*.—The same, somewhat later, showing various retractions and shiftings of quadruple internodal traces.

FIG. 15.—Part of P.M.C. at same stage as preceding.

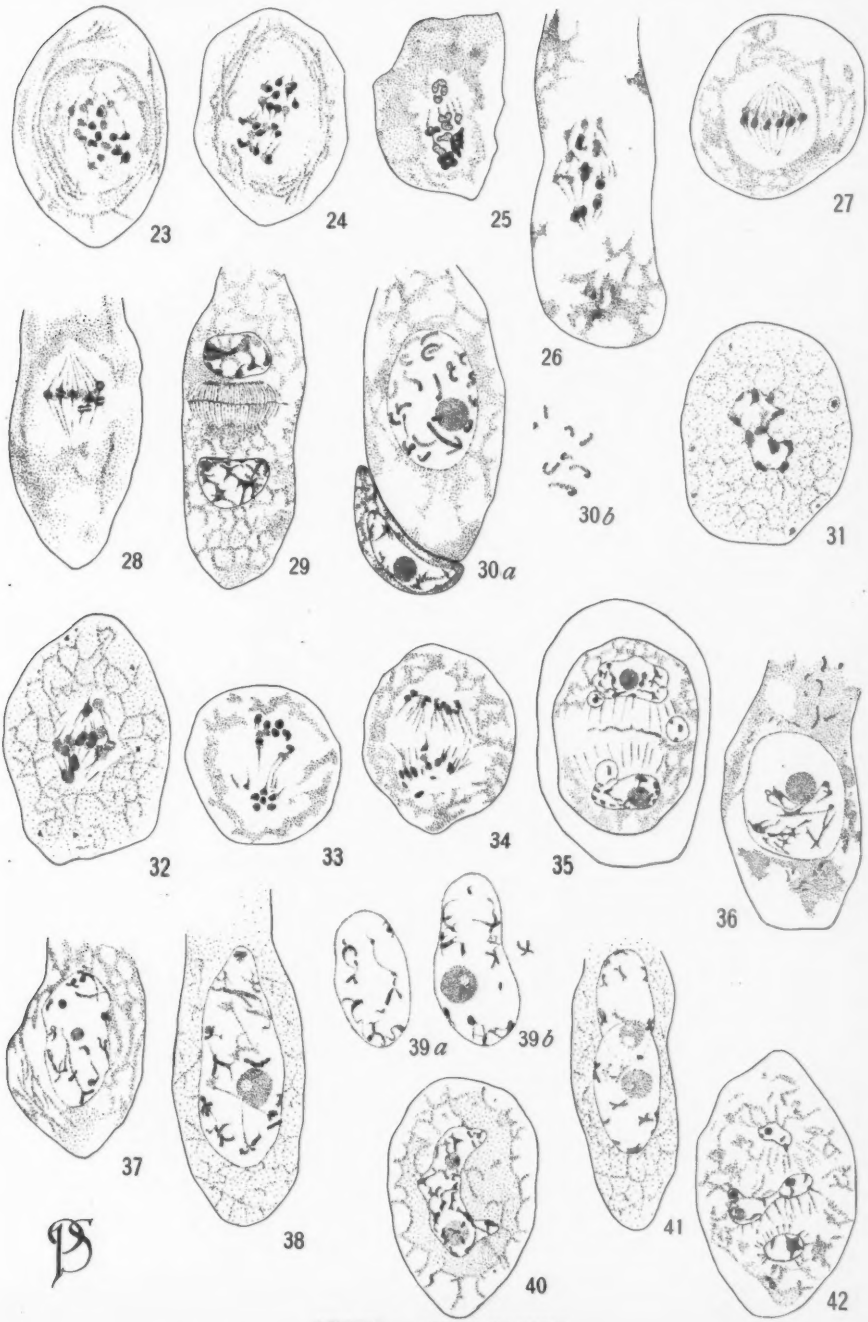
FIG. 16.—Complete P.M.C., showing beginning of end to end synaptic pairing of twenty-six univalent cuboids.

FIG. 17.—Late orientation stage in sequence *A*, E.S.M.C. showing synapsis completed.

FIG. 18.—Same for P.M.C.



SEARS on TARAXACUM



SEARS on TARAXACUM

FIG. 19.—P.M.C. at metaphase, sequence *A*, showing narrow heterotypic spindle.

FIG. 20.—P.M.C. showing heterotypic anaphase of sequence *A*.

FIG. 21*a*, *b*.—Complementary sections of E.S.M.C. anaphase, masked but doubtless sequence *A*.

FIG. 22.—Second, true homotypic metaphase of sequence *A* in P.M.C., showing thirteen chromosomes at plate.

FIG. 23.—P.M.C. orientation in sequence *B*, pairing somewhat delayed; nuclear membrane disappearing.

FIG. 24.—P.M.C. showing beginnings of delayed synaptic pairing in sequence *B;* membrane quite gone (cf. fig. 16).

FIGS. 25, 26.—Two different E.S.M.C.'s somewhat further along than fig. 24; such stages grade insensibly into sequence *C*.

FIG. 27.—P.M.C. showing wide metaphase spindle characteristic of sequence *B*.

FIG. 28.—E.S.M.C. in ameiotic metaphase of sequence *B;* synaptic mates end to end, perpendicular to spindle axis, giving quantitative division along line of prophase split.

FIG. 29.—Telophase of first E.S.M.C. division, probably sequence *B*.

FIGS. 30*a*, *b*.—Second division, purely somatic, following ameiosis in E.S.M.C; note breakdown of apical daughter and presence of about twenty-six homogeneous segments.

FIG. 31.—Portion of P.M.C. showing disturbance in orientation due to premature elongation of nucleus; beginning of sequence *C*.

FIG. 32.—Similar portion, further along; pairing about complete, but spindle defective (cf. fig. 25).

FIG. 33.—P.M.C. in sequence *C*, synapsis virtually complete but chromosomes unequally sequestered at poles without orientation or metaphase.

FIG. 34.—P.M.C. in sequence *C*, chromosomes sequestered with little or no synapsis.

FIG. 35.—P.M.C. in irregular telophase, probably of sequence *C*, showing organization of small supernumerary nuclei.

FIG. 36.—E.S.M.C., late segmentation stage to show gradation into sequence *D* or amitosis.

FIG. 37.—E.S.M.C. showing beginnings of nuclear elongation during late segmentation.

FIG. 38.—The same, showing persistence of internodes between *X*-shaped chromosomes and origin of latter by persistence of prophase split.

FIGS. 39*a*, *b*.—Complementary E.S.M.C. sections, showing more than twenty split chromosomes.

FIG. 40.—Beginning amitotic division (sequence *D*) in P.M.C.

FIG. 41.—Amitotic constriction of sequence *D* in E.S.M.C.; note chromosome form.

FIG. 42.—Amitotic irregularities in P.M.C., showing also chromatin extrusion.

ANNULARIA WITH PALEOSTACHYA FRUIT

EDA M. ROUND

(WITH TWO FIGURES)

Among the most common fossil plants in Rhode Island are Annulariae, thought to be allied to the modern *Equisetum*. While the Annulariae or leafy shoots are seldom found attached to their supposedly Calamitean stems, the trunks of these ancient cryptogams are often seen in the coal strata and occasionally prove to be of large size. Much more rare, however, are the fruiting stalks of these primitive plants, one specimen of which has appeared from the coal shales of Rhode Island. This fossil is regarded by the writer as a new species of *Annularia*, both from the character of its foliage and the nature of its fruit.

Annularia clarkii, n. sp.—In considering the affinities of the leafy shoots of *Annularia clarkii* one may cite *Asterophyllites lentus* Dawson.[1] The Canadian material, however, is so fragmentary that correlation therewith is questionable. The fertile stalks of *Annularia clarkii* resemble in many ways *Paleostachya* (*Volkmannia*) *gracilis* Renault,[2] especially in the position of the sporangia, which appear to be borne in the axils of the leaves. The sporangiophores of the European species, however, are shorter and less stout than those of the Rhode Island plant (figs. 1, 2).

In a recent publication[3] statements are made to the effect that Calamariae, to which the Annulariae supposedly belong, show four main types of fruiting. The first includes *Calamostachys*, forms in which the cones are made up of fertile and sterile parts, the sporangiophores being placed midway between the leafy bracts. The second or *Paleostachya* type consists of cones, the fertile parts of which are borne in the axils of the sterile bracts. The third or *Cingularia* type is characterized by sporangiophores borne just under

[1] Bull. Nat. Hist. Soc. N.B. **6**:247. 1910.

[2] RENAULT, B., Autun **2**:75. *pl. 29. figs. 1–7.* 1893.

[3] SCOTT, D. H., Studies in fossil botany. Ed. 3. Vol. I. p. 43. 1920.

the sterile whorls. The fourth has a sporangiophore without bracts, or arranged as in the modern *Equisetum*. The same author states

FIG. 1 FIG. 2

FIGS. 1, 2.—Fig. 1, *Annularia clarkii:* photograph, natural size; fig. 2, *Annularia clarkii:* drawing, ×3.

further that "in all cases where fructifications have been referred to *Annularia* . . . . they have been proved to be of the *Calamostachys* type."

Another English author[4] of earlier date, however, states the situation less sweepingly, as follows:

> It would appear . . . . that the *Annularia* type of branch usually bears cones which conform to the genus *Calamostachys* (*Stachannularia*); . . . . But this rule is not constant, and we are not in a position to speak of cones of a particular type as necessarily characteristic of definite types of Calamitean shoots.

BERRY[5] has stated the problem in a still more positive way as follows: "Some of the *Paleostachya* cones are of large size and they are usually associated with *Annularia* type of foliage." This opinion is further borne out by the characteristics of the present species, *Annularia clarkii*. The illustrations of this fossil reveal several fruiting cones attached to their leafy shoot, the lower right hand one being sufficiently distinct to show that the sporangiophores spring from the axils of the leaves. This fact places the specimen in the *Paleostachya* group, and refutes the contention by SCOTT that Annulariae have always been characterized by *Calamostachys* types of fruiting. The following description of *Annularia clarkii* is offered:

Verticilli 10–12 foliorum circa quemque nodum; quodque folium linearium 4 cm. vel longum paullum, apices acuti, mediae costae apparent; fructus ramus quemque nodum quasi 4.5 cm. longus, sporangio-phoreae quasi 1 mm. longae. Duae fruges annectae sunt alterno lato stili curvati. Hic ultimus in axilla curtae bractae linearae est, quae fructum superarcuant. Plurimi stili sporosi ex unico nodo enascuntur.

Whorls of 10–12 leaves at each node; each leaf linear, 4 cm. or less in length, apex acute, midrib present; fruiting branch at each node about 4.5 cm. long, the sporangiophore about 1 mm. long. Two spores are attached on either side of a curved stalk. The latter is in the axil of linear bracts which overarch the fruit. Several spore-bearing stalks seem to spring from a single node.

Sterile fossils of this species are very common from the Pawtucket, Valley Falls, and Portsmouth sections of Rhode Island, and may be found in the Brown University collection. The fruited specimen used as the basis of this article is now a part of the Roger Williams Park Museum collection, Providence, Rhode Island.

[4] SEWARD, A. C., Fossil plants. Vol. I. p. 364. 1878.

[5] BERRY, E. W., Paleobotany: A sketch of the origin and evolution of floras. p. 319. 1920.

CURRENT LITERATURE

NOTES FOR STUDENTS

The unarmored Peridiniales.—Excepting only the diatoms, no group of unicellular organisms is of such fundamental importance in the biology of the seas as the peridines, and there are few groups concerning which our knowledge is so unsatisfactory. This is particularly true of the unarmored forms, which are not only apt to be ruined in collecting, but, if they survive the plankton net, are destroyed by the preservatives used and usually remain recognizable for only a short time under the conditions speedily developed in a jar of sea water or on a microscopic slide. For these reasons all who have occasion to study the minute life of the ocean will heartily welcome the appearance of the splendid monograph by KOFOID and SWEZY.[1] About one-fifth of the text is devoted to topics of a general nature, the remainder to the detailed descriptions of genera and species. Among the topics discussed are the general and comparative morphology of the group, life cycles, physiology, including short but pregnant discussions of nutrition and luminescence, and finally evolutionary development and distribution. The authors take marked exception to WEST'S statement that over 80 per cent of the peridines are "true vegetable organisms with a holophytic nutrition," declaring on the contrary that "the number actually containing chromatophores is relatively small throughout the entire Dinoflagellata." In spite of this, however, the great abundance of some of the forms characterized by undoubted holophytic nutrition insures that the group will continue to be of as great interest to the botanist as to the zoologist.

The authors regard the Peridiniales as a monophyletic group, derived from a cryptomonad ancestry, and describe a new genus, *Protodinifer*, which is one of the simplest known peridines. One line of development, starting near some such form, may have given rise to the *Haplodinium*, *Exuviella*, *Prorocentrum* series; another would lead to the simpler Gymnodiniaceae, from which coordinate lines of development lead on the one hand to the higher unarmored forms, culminating in such elaborately organized genera as *Pouchetia* and *Erythropis*, and on the other hand to the thecate forms, such as *Ceratium*.

The systematic treatment includes descriptions of 223 species, distributed among 16 genera, of which *Protodinifer*, *Gyrodinium*, *Torodinium*, *Pavillardia*, *Protopsis*, *Nematodinium*, and *Proterythopsis* are new. Of these, *Torodinium*

[1] KOFOID, C. A., and SWEZY, OLIVE, The free-living unarmored Dinoflagellata. Memoirs Univ. Cal. **5**: viii+562. *pls.* 12 (colored). *figs.* 388. Univ. Cal. Press. Berkeley. 1921.

and *Protopsis* include in part previously described species, and *Gyrodinium* replaces SHÜTT'S genus *Spirodinium* which is preoccupied zoologically. The other new genera are established to contain newly discovered species.

A few minor typographical errors occur, but in general the work is remarkably free from them. The text figures are irregularly distributed and frequently many pages from the references to them. It would greatly facilitate the use of the book if the references were by page instead of merely to the figure. These are trifling defects, however, in a work of such unusually permanent value. It is to be confidently expected that its publication will prove a distinct stimulus to further study in a field where there is still much to be learned.—G. W. MARTIN.

A new type of nuclear division.—CHATTON,[2] in a brief but significant paper throws new light on the peculiar nuclear phenomena in the Peridiniales described by LAUTERBORN, JOLLOS, BORGERT, and in his own earlier contributions. His studies were made on species of *Syndinium*, which live parasitically in the body cavity of copepods, especially favorable material because of the small number of chromosomes. These have previously been described as ten somewhat curved filaments from a single pole like the ribs of an umbrella. Further study has shown that this structure is in reality composed of five V-shaped chromosomes with very sharp bends, with the points of the V's converging at the pole. Cleavage takes place throughout the length of the chromosomes, so that ten V-shaped daughter chromosomes are formed. Five of these remain grouped about the original pole; the points of the remaining five become centered about a new pole which is at first close to the original one, then gradually moves away. In some species the cleavage is completed at once; in others the daughter chromosomes remain united at their tips and turn as upon a hinge, so that a bipolar spindle-shaped structure (not a true achromatic spindle) is formed, composed of the two series of V-shaped chromosomes converging at either end. The chromosomes then break apart, and it is this separation which was formerly interpreted as a transverse division of chromosomes, when it is in reality merely the final separation of chromosomes previously formed by longitudinal fission. Nothing resembling a true spindle was seen.

Ordinarily no resting stage appears to occur, but it was observed in certain cases where the development of the peridine had been inhibited by some factor, as, for example, the presence of another parasite. In such cases the nucleus appeared to be composed of a large number of microsomes grouped around a central nucleolus.

A division of this kind, while distinctly simpler than one taking place in connection with the usual achromatic structures, would appear to be quite as

[2] CHATTON, EDOUARD, Sur un mecanisme cinetique nouveau. La mitose syndienne chez les Peridiniens parasites plasmodiaux. Compt. Rend. Acad. Sci. **173**: 859–862. *fig. 1.* 1921.

effective in securing an exact distribution of the chromatin. The author proposes for it the special term "syndinial mitosis," and expresses the opinion that further study of the free peridines will show that nuclear division in such forms is also of this type.—G. W. MARTIN.

Cytology of Porphyra.—Since a cytological study of the Bangiales might throw light upon the much discussed but little investigated problem as to whether this group is primitive or reduced, whether it should stand at the beginning or at the end of the red algae, ISHIKAWA[3] fixed material and has described several critical stages in the life history of *Porphyra tenera*, a characteristic genus of the order.

The cell wall shows no cellulose reaction, but responds to tests for pectic substances. The large stellate chromatophore contains one conspicuous pyrenoid which has often been mistaken for a nucleus, the real nucleus (only 1.5–2 μ in diameter) being hard to detect in living material, although easy to see in well stained preparations, where it appears as a black globule with no structural differentiation. At division the nucleus elongates, and splits by longitudinal fissures into three filaments which constrict in the middle, so that three pieces go to each pole. The process looks like that described for some Cyanophyceae, especially *Synecocystis*. The figures show neither nucleolus nor nuclear membrane, so that the type appears to be very primitive. The antheridium consists of 64 or 128 cells, and the spermatium has a chromatophore and a group of three chromosomes without any nuclear membrane. The carpogonium is slightly prominent at both ends, the prominence constituting a rudimentary trichogyne. Spermatia were found attached to the trichogyne, but the actual process of fertilization was not observed, nor was there any study of the first and second divisions of the zygote. It seems reasonable, however, to suppose that reduction of chromosomes occurs during these divisions, as in many other algae.

ISHIKAWA would regard Bangiales as a connecting link between the Cyanophyceae and the Florideae, a conclusion which is helped by the fact that *Porphyridium*, a genus sometimes placed in one group and sometimes in the other, has no sexual reproduction. So far as pigments are concerned, some of the Rhodophyceae have phycocyan and some of the Cyanophyceae have phycoerythrin.

Although a careful investigation of the whole life history of several members of the Bangiales is desirable, it seems probable that any future study of the group will confirm ISHIKAWA's conclusions.—C. J. CHAMBERLAIN.

Ecology of Urtica dioica.—In an interesting study of the factors which locally limit the distribution of the common nettle, *Urtica dioica*, OLSEN[4]

[3] ISHIKAWA, M., Cytological studies on *Porphyra tenera*. Bot. Magazine Tokyo **35**: 206-218. 1921.

[4] OLSEN, CARSTEN, The ecology of *Urtica dioica*. Jour. Ecol. **9**: 1–18. *pl. 1.* 1921.

states that it is abundant in Denmark, growing mostly in large close communities. Its gregarious habit is due to the activities of horizontal rhizomes, which also account for the sharpness of the boundaries separating it from other vegetation. It thrives well in full light, and in shade up to 5–10 per cent of open daylight, the greatest vigor being shown in 10–20 per cent of full illumination. A moderate amount of soil moisture also seems necessary to meet its requirements; but while OLSEN has determined the percentage of soil moisture in the habitats under consideration, he has not attempted to determine the growth water available.

By several authors *Urtica dioica* has been placed among nitrogen-requiring plants, and the principal feature of this investigation was the experimental examination of this quality. Microchemical tests showed that the plants themselves always contained considerable nitrates, especially in the stems, rhizomes, and roots. Soil samples were then taken at a depth at which the roots of the plant abound, in twenty localities, some within and others without the nettle communities. The nitrate content of such samples was determined, both at once after they were taken, and again after the soil had been kept moistened in a jar at 18° C. for twenty-five days. This procedure was in order to obtain an expression of the nitrifying power of the soil. These data were tabulated, together with the hydrogen-ion concentration, the percentage of soil moisture, and the light value of the locality. The table makes it evident that the only factor varying directly with the presence of *Urtica dioica* is the nitrate content of the soil.

Experimental cultures of the plant were made, using portions of rhizomes planted in washed sand and watered with nutrient solutions containing varying amounts of nitrates. Growth was in direct proportion to the relative amounts of available nitrogen; hence the conclusion was reached that *Urtica dioica* in nature is able to make sufficiently vigorous growth to enable it to compete successfully with other vegetation only where there is a relatively large amount of nitrogen in available form present in the soil. The experiment also showed that nitrification proceeds in soils showing an acid reaction as high as $P_H = 3.6$, and that ammonium used as a source of nitrogen was toxic to the nettle.—GEO. D. FULLER.

Pink root of onions.—Another of the many diseases directly attributed by farmers to alkali in the soil has recently been shown to be due to a parasite, as a result of investigations carried on in the Texas Experiment Station.[5] Isolation and inoculation studies have definitely connected a species of *Fusarium*, tentatively called *F. malli*, with the pink root disease of onions. Over twenty-five species of fungi were found in diseased plants, including several species of *Fusarium*, and the association of some of these fungi with *F. malli* increased the virulence of the latter. All varieties of onions and garlic

[5] TAUBENHAUS, J. J., and MALLY, W., Pink root disease of onions and its control in Texas. Texas Agric. Exp. Sta. Bull. 273. pp. 42. *figs.* 3. 1921.

inoculated appeared to be more or less susceptible to the pink root organism, but other liliaceous plants, such as *Funkia*, *Tulipa*, *Calla*, *Iris*, and *Lilium* were immune. Pink root of onions has been observed in California, Iowa, Wisconsin, New York, and the Bermuda Islands. In Texas it seriously threatens the industry of growing onions for the early northern markets, which industry has become an important one. Losses vary from $150 to $400 per acre.

Symptoms of pink root include yellowing of the roots, followed by their pink discoloration, drying, and death. The bulb exhausts its energy in producing new roots. Alkali soil, deficiency in nitrogen and humus, excessive temperatures, eel worm and thrips attacks are factors favoring the disease. The seed is not a carrier, but onion "sets," both dry and green, may harbor the causal fungus. Suggested control methods include the use of virgin soil for seed bed and field plantings, steam or formaldehyde disinfection of seed beds known to contain the pink root fungus, rotation of crops, the use of quickly acting fertilizers, careful use of tools, and various cultural practices favoring continued growth of the crop. An attempt to control nematodes by adding cyanimide to the soil failed because the amount required to affect nematodes killed the crop.—J. G. BROWN.

Carbon nutrition.—Storage rot fungi of the sweet potato have been investigated by WEIMER and HARTER,[6] who find that seven of eight species causing rot can utilize glucose as a source of carbon. Five of them are able to increase the acidity of the culture medium, and certain species increased the osmotic concentration of the substratum. The glucose is utilized partly as a source of energy, partly in producing mycelium, and perhaps in still other ways. The respiratory activity of these organisms has been studied by the same authors,[7] who used the amount of CO_2 set free as the measure of the carbohydrate used in this process. *Penicillium* sp., *Botrytis cinerea*, and *Sclerotium bataticola* grew slowly, produced relatively large amounts of dry material, consumed nearly all of the glucose, and produced CO_2 most freely. The other species grew more rapidly, but produced comparatively small amounts of CO_2 and did not consume all the glucose. The economic coefficient was found unusually high in two species. *Fusarium acuminatum* required 17.11 G. and *Mucor racemosus* 22.86 G. glucose for each gram of dry matter grown. The CO_2 set free is not equal to the theoretical amount that could have formed from the sugar consumed. Some of the sugar evidently was not completely respired, as alcohol and acids appeared in some of the culture solutions.—C. A. SHULL.

Transmission of potato wilts.—Among the various wilts which are responsible for heavy losses sustained by potato growers are those due to attacks of

[6] WEIMER, J. L., and HARTER, L. L., Glucose as a source of carbon for certain sweet potato storage rot fungi. Jour. Agric. Res. **21**: 189–210. 1921.

[7] ———, Respiration of sweet potato storage rot fungi when grown on a nutrient solution. Jour. Agric. Res. **21**: 211–226. 1921.

Fusarium oxysporium and *Verticillium albo-atrum*. In order to determine the degree of transmission of these wilts through seed tubers, McKAY[8] has carried on experiments with numerous varieties of potatoes for four years. *Verticillium albo-atrum* occurs somewhat more extensively in small tubers than in medium-sized ones, 30–50 per cent of the crop grown from infected seed tubers being diseased with *Verticillium* wilt, as shown by cultures. *Fusarium oxysporium* is transmitted in a lesser degree, and it appears to be capable of remaining virulent in the soil for several years after the production of a crop of potatoes. Vascular discoloration is an unreliable index of *Verticillium* infection, since approximately 7–17 per cent of cultured tubers which produced the fungus show no discoloration, and 55 per cent of the tubers which show brown vascular discoloration give no organism parasitic for the potato. Although the discoloration occurs at the stem end of the tuber, stem-end seed pieces give no more disease than eye-end pieces of the same infected tuber. Numerous species of *Fusarium* and other fungi mostly saprophytic in nature appear in cultures of wilt diseased tubers.—J. G. BROWN.

Colloidal hydration.—In two recent papers MACDOUGAL[9, 10] discusses the effects of bases, salts, and other substances on the hydration capacity of prepared colloidal bodies and masses of vegetable cells. In a previous paper[11] he had reported that 0.01 N hydroxides retard the hydration of colloids, and suggested that the chief function of the base forming metals required by plants might be the restricting or limiting of the hydration capacity of the living protoplasm. He now finds that when concentrations of 0.001 to 0.0001 M solutions of chlorides and nitrates, and 0.001 to 0.0001 N hydroxides are used, concentrations comparable to those occurring in living cells, the hydration is increased and not restricted. He therefore reinterprets the function of the metallic elements as accelerators of hydration and growth. Correction is also made regarding the effects of HCl. At a P_H value of 4.2 the acid is now shown to cause more swelling than water. Some interesting studies of the hydration of roots of different ecological types, and of roots grown in different types of soil are reported. In general he concludes that all substances which are known to facilitate growth in plants will at appropriate concentrations increase the hydration capacity in some of the colloidal objects tested.—C. A. SHULL.

Vertical distribution of Fucus.—*Fucus* has long been regarded as characteristic of the zone of tidal play, largely because of its high light requirement

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[8] McKAY, B. M., Transmission of some wilt diseases in seed potatoes. Jour. Agric. Res. **21**:821–847. 1921.

[9] MACDOUGAL, D. T., Water deficit and the action of vitamines, amino-compounds, and salts on hydration. Amer. Jour. Bot. **8**:296–302. 1921.

[10] ———, The action of bases and salts on biocolloids and cell masses. Proc. Amer. Phil. Soc. **60**:15–30. 1921.

[11] ———, Growth in organisms. Science **49**:599–605. 1919.

and its capacity for exposure to the air. GAIL has made some exact studies to determine the controlling factors in distribution.[12] High light requirement is well shown by the fact that the average vertical distance occupied by *Fucus* on south slopes is over 2 m., while on north slopes it is less than one-third of a meter. North exposures with a high shore line have no *Fucus* at all, and there is little or no *Fucus* under overhanging trees. Careful experimental study showed that mature *Fucus* plants are more resistant to low light intensities than are sporelings, that reduced light intensities cause the death of well grown *Fucus* plants 1 m. below the water surface, and that reduced light causes the death of oospores and sporelings when planted more than 3 dm. below the water surface. Well grown *Fucus* plants receiving less than one-fourth total light become darker in color, and decomposition takes place. From these considerations it is properly concluded that light is a controlling factor in determining the lower limit of *Fucus*.—H. C. COWLES.

Vegetation of the Dry Tortugas.—The Tortugas are the westernmost of the Florida keys, and are the seat of a marine laboratory of the Carnegie Institution. While engaged in other work, BOWMAN took occasion to make a detailed study of the distribution and special ecology of the vegetation of the Dry Tortugas.[13] After brief statements on the geology and the climatic conditions, the author presents a general sketch of the vegetation, which speaking broadly belongs entirely to the strand flora. Even *Rhizophora* is lacking in the sense of an association, because of the xerophytism of the conditions. Four communities are recognized, dominated respectively by *Uniola paniculata*, *Suriana maritima*, *Opuntia Dillenii*, and *Chamaesyce buxifolia*. A detailed account then follows of the special vegetation of each of the eight keys that make up the group. Of especial interest is the author's comparison of the vegetation of the islands in 1915 and 1916 with their vegetation in 1904, as reported by LANSING.—H. C. COWLES.

Scrophulariaceae and Orobanchaceae.—BOESHORE[14] has reached the conclusion that the Orobanchaceae represent an extreme offshoot from the Scrophulariaceae. This conclusion is based upon a detailed study of the roots, stems, leaves, flowers, and seeds of both families. From a review of these details, the author concludes that there is ample evidence "to show that direct and distinct continuity can be established from non-parasitic through semi-parasitic Scrophulariaceae to the most degraded parasites of the family, and that these again show direct continuity with the still more degraded

[12] GAIL, FLOYD W., Some experiments with *Fucus* to determine the factors controlling its vertical distribution. Publ. Puget Sound Biol. Sta. **2**:139–151. 1918.

[13] BOWMAN, H. H. M., Botanical ecology of the Dry Tortugas. Carnegie Inst. Washington Publ. **252**:109–138. *pls. 6. figs. 7.* 1918.

[14] BOESHORE, I., The morphological continuity of Scrophulariaceae and Orobanchaceae. Contrib. Bot. Lab. Univ. Penn. **5**:139–177. *pls. 12–16.* 1920.

and condensedly parasitic types of Orobanchaceae." The author cites the genera that represent the various stages in this series, and also describes the progressive changes in the various structures.—J. M. C.

Permeability.—The permeability of *Laminaria agardhii* as affected by anions from various inorganic and organic salts of sodium has been measured by RABER,[15] who used OSTERHOUT's electrical conductivity method. All of the anions increase permeability, following in a general way the HOFMEISTER series. The anions arrange themselves by their effects into several groups: thus the monovalent, bivalent, and trivalent groups can be recognized by the quantitative difference in permeability change with members of each group. The tetravalent anion, $Fe(CN)_6$, did not produce a fourth group, but this is explained as due to low concentration of the salt. The author believes that the effects of anions on permeability depend upon the valency of the anion, regardless of whether the salts are organic or inorganic.—C. A. SHULL.

A maritime species.—Following the methods employed by BONNIER of dividing individual plants and growing the resulting halves under different climatic conditions, DANIEL[16] in 1902 separated plants of *Asphodelus luteus* growing at Rennes (France) and planted portions of them in a seaside garden at Erquy. As a result of the maritime climate such striking changes resulted in the general form of the plant, in the branching habit of the inflorescence, and in other structural features that at present the seaside forms are sufficiently distinct to be regarded as a distinct species. This derived species he has named *Asphodelus luteoides*. This he believes to be the first recorded instance of maritime conditions transforming a plant to such an extent that the resulting form is entitled to specific rank.—G. D. FULLER.

Animal burrows an ecological factor.—On some small islands in the outer archipelago of Stockholm possessing a humid oceanic climate ROMELL[17] reports that voles eating the grass roots within their burrows upset the ecological equilibrium and cause strips of *Sphagnum* to replace the turf. The irregular mosaic thus formed, however, is not permanent, as the *Sphagnum* seems unable to resist the invasion of the grass.—GEO. D. FULLER.

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[15] RABER, O. L., A quantitative study of the effect of anions on the permeability of plant cells. I. Jour. Gen. Physiol. **2**:535–539. 1920; II. Amer. Jour. Bot. **8**:366–368. 1921.

[16] DANIEL, LUCIUS, Obtention d'une espèce nouvelle d'Ashphodèle par l'action du climat marin. Rev. Gen. Bot. **33**:225–237, 316–327, 357–371, 420–436. *pls. 3. figs. 12.* 1921.

[17] ROMELL, L. G., Voles as a factor in plant ecology. Svensk Bot. Tidsk. **15**:43–45. 1921.

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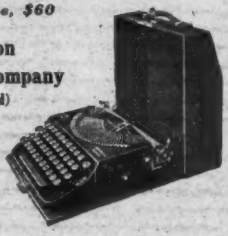

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